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(54) Title: IMMUNOGENIC AND THERAPEUTIC COMPOSITIONS *FOR STREPTOCOCCUS PYOGENES*

(57) Abstract: Group A streptococcal (GAS) antigens useful for providing immunity against *pyogenes* infection.

IMMUNOGENIC AND THERAPEUTIC COMPOSITIONS FOR *STREPTOCOCCUS PYOGENES*

[01] This application claims the benefit of and incorporates by reference co-pending provisional applications Serial No. 60/616,854 filed October 8, 2004; Serial No. 60/652,736 filed February 15, 2005; Serial No. 60/701,121 filed July 21, 2005; and Serial No. 60/705,209 filed August 4, 2005.

[02] This application incorporates by reference the contents of each of four CD-ROMs, each of which contains an identical 1.75 KB file labeled "sequence listing.txt" and containing the sequence listing for this application. The CD-ROMs were created on October 11, 2005.

FIELD OF THE INVENTION

[03] This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Streptococcus pyogenes* and their use in immunization.

BACKGROUND OF THE INVENTION

[04] Group A streptococcus ("GAS," *S. pyogenes*) is a frequent human pathogen, estimated to be present in between 5-15% of normal individuals without signs of disease. An acute infection occurs, however, when host defenses are compromised, when the organism is able to exert its virulence, or when the organism is introduced to vulnerable tissues or hosts. Related diseases include puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotizing fasciitis, myositis, and streptococcal toxic shock syndrome.

[05] GAS bacteria are gram positive, non-spore forming coccus-shaped bacteria which typically exist in chains or in pairs of cells. GAS bacteria are subdivided according to serotyping based on a large, highly variable cell surface antigen call the M protein. Lancefield, J. Exp. Med. 47, 9-10, 1928; Lancefield, J. Immunol. 89, 307-13, 1962. DNA sequencing of genes encoding M proteins has become the most common method of

determining GASM types (emm sequence types). To date 124 different M types have been identified; 22 of these types were identified between 1995 and 1998 (Facklam *et al.*, Clin. Infect. Dis. 34, 28-38, 2002). M1, M28, M12, M3, M11, and M6 are among the most prevalent GAS types worldwide. Li *et al.*, Infect. Dis. 188, 1587-92, 2003; O'Brien *et al.*, Clin. Infect. Dis. 35, 268-76, 2002.

[06] Although *S. pyogenes* infections can be treated using antibiotics, there is a need in the art for prophylactic vaccines to prevent the onset of disease, as well as for additional therapies for treating *S. pyogenes* infections.

BRIEF DESCRIPTION OF THE FIGURES

[07] FIG. 1. Alignment of the amino acid sequences of GAS40 proteins from GASM strains SF370, 2580, 3280, 3348, 3789, and 2913 (SEQ ID NO:17) 2634 (SEQ ID NO:18), 2726 (SEQ ID NO:19), 2721 (SEQ ID NO:20), 3040 and 3135 (SEQ ID NO:21), 2722 (SEQ ID NO:22), 2728 (SEQ ID NO:23), 4883 (SEQ ID NO:24), 2724 (SEQ ID NO:25), 2894, 3650, 5529, and 3776 (SEQ ID NO:26), 2720 (SEQ ID NO:27), 2725 (SEQ ID NO:28), 4538 (SEQ ID NO:29), 5531 (SEQ ID NO:30), 5481 (SEQ ID NO:31), 4959 (SEQ ID NO:32), D2071 (SEQ ID NO:33), 4436 (SEQ ID NO:34), 2727 (SEQ ID NO:35), 2719 (SEQ ID NO:36), 5455 (SEQ ID NO:37), 5476 (SEQ ID NO:38), 4088 (SEQ ID NO:39), MANFR10394 (SEQ ID NO:40), M8232 (SEQ ID NO:41), M315 (SEQ ID NO:42), and SS1 (SEQ ID NO:43). FIG. 1A, amino acids 1-50; FIG. 1B, amino acids 51-100; FIG. 1C, amino acids 101-150; FIG. 1D, amino acids 151-200; FIG. 1E, amino acids 201-250; FIG. 1F, amino acids 251-300; FIG. 1G, amino acids 301-350; FIG. 1H, amino acids 351-400; FIG. 1I, amino acids 401-450; FIG. 1J, amino acids 451-500; FIG. 1K, amino acids 501-550; FIG. 1L, amino acids 551-600; FIG. 1M, amino acids 601-650; FIG. 1N, amino acids 651-700; FIG. 10, amino acids 701-750; FIG. 1P, amino acids 751-800; FIG. 1Q, amino acids 801-850; FIG. 1R, amino acids 851-873.

[08] FIG. 2. Results of FACS analysis demonstrating that GAS40 proteins are exposed on the cell surface of strains of different M types.

[09] FIG. 3. Results of FACS analysis demonstrating that antisera directed against native GAS40 protein detects GAS40 protein on the cell surface of strains of different M types (strains DSM2071, 2634, a hypocapsulated mutant of DSM2071, 2727, SF370, 2720, 3789, 2725, 2580, 2894, 2728, 2913, 2726, 3348, and 3280).

[10] FIG. 4A-B. Results of FACS analysis demonstrating that antisera directed against a "GST-GAS40" antigen detects GAS40 protein on the cell surface of strains of different M types. FIG. 4A, strains 3789, 4883, a hypocapsulated mutant of DSM2071, 5476, SF370, DSM2071, 2720, 2723, 2728, 2724, 2580, 2725, 2719, 2726, 3776. FIG. 4B, strains 4436, 2721, 4959, 2727, 5468, 3650, 2634, 4088, 4538, 2722.

[11] FIG. 5A-B. Results of FACS analysis demonstrating that antisera directed against a GAS40a antigen detects GAS40 protein on the cell surface of strains of different M types. FIG. 5A, DSM2071, SF370, 2721, 3280, 2728, 3789, a hypocapsulated mutant of DSM2071, 4883, 5476, 2725, 2720, 2726, 2723, 2728, 2724, and 2580; FIG. 5B, 2719, 5468, 3776, 2634, 4436, 2721, 4959, 2727, 3650, 4088, 4538, and 2722.

[12] FIG. 6A-B. Results of FACS analysis demonstrating that antisera directed against a GAS40aCH antigen detects GAS40 protein on the cell surface of strains of different M types. FIG. 6A, strains DSM2071, 3280, 2721, 3789, 2728, 4883, a hypocapsulated mutant of DSM2071, 5476, SF370, 2720, 2723, 2580, 2724, 2719, 2725, 3776, 2726, 4436, 2728, and 4959; FIG. 6B, strains 5468, 4088, 2634, 4538, 2721, 2722, 2727, and 3650.

[13] FIG. 7. Results of FACS analysis demonstrating that antisera directed against a GAS40/GAS117 hybrid antigen detects GAS40 protein on the cell surface of strains of different M types (strains 2720, 2726, 2725, 3280, 2580, 2728).

[14] FIG. 8. Results of FACS analysis demonstrating that antisera directed against a GAS117/GAS40 hybrid antigen detects GAS40 protein on the cell surface of strains of

different M types (strains DSM2071, 2634, a hypocapsulated mutant of DSM2071, 2727, 3789, 2720, SF370, 2725, 2580, 2894, 2728, 2913, 2726, 3348, 3280).

[15] FIG. 9A-B. Results of FACS analysis demonstrating that antisera directed against a GAS40aRR antigen detects GAS40 protein on the cell surface of strains of different M types. FIG. 9A, strains DSM2071, 3280, 2721, 4789, 2728, 4883, a hypocapsulated mutant of DSM2071, 5476, SF370, 2720, 2723, 2580, 2724, 2719, 2725, 3776, 2726, 4436, 2728, 4959; FIG. 9B, strains 5468, 4088, 2634, 4538, 2721, 2722, 2727, 3650.

[16] FIG. 10A-B. Results of FACS analysis demonstrating that antisera directed against a GAS40aNH antigen detects GAS40 protein on the cell surface of strains of different M types. FIG. 10A, strains DSM2071, 3280, 2721, 3789, 2728, 4883 a hypocapsulated mutant of DSM2071, 5476, SF370, 2720, 2723, 2580, 2724, 2719, 2725, 3776, 2726, 4436, 2728, 4959; FIG. 10B, strains 5468, 4088, 2634, 4538, 2721, 2722, 2727, 3650.

[17] FIG. 11A-B. Results of FACS analysis demonstrating that antisera directed against a GAS40aRRNH antigen detects GAS40 protein on the cell surface of strains of different M types. FIG. 11A, strains DSM2071, 3280, 2721, 3789, 2728, 4883, a hypocapsulated mutant of DSM2071, 5476, SF370, 2720, 2723, 2580, 2724, 2719, 2725, 3776, 2726, 4436, 2728, 4959; FIGS. 11B, strains 5468, 4088, 2634, 4538, 2721, 2722, 2727, 3650.

[18] FIG. 12A-B. Diagram of expression vectors and recombinant GAS antigens. FIG. 12A, expression vectors pET-21+ and pGEX; FIG. 12B, encoded recombinant proteins.

[19] FIG. 13. Schematic view of mouse model.

[20] FIG. 14. Mouse model results.

[21] FIG. 15. Schematic view of GAS40 structure.

[22] FIG. 16. Western blots showing expression of GAS40 in different GAS serotypes.

[23] FIG. 17. FACS pictograms showing surface expression of GAS40.

[24] FIG. 18. Photomicrographs showing distribution of GAS40 on the bacterial cell surface.

[25] FIG. 19. Graph illustrating bactericidal properties of anti-GAS40 antibodies.

[26] FIG. 20. Graph illustrating opsonization properties of anti-GAS40 antibodies.

[27] FIG. 21. Schematic view of GAS40 domains.

[28] FIG. 22. Graph illustrating time course survival results for mice immunized with GAS40N (SEQ ID NO:930).

[29] FIG. 23. FACS data demonstrating that GAS40 is surface exposed across different M strains.

[30] FIG. 24. Western blots and FACS graphs demonstrating that the four monoclonal antibodies tested do not bind to a GAS40N epitope.

[31] FIG. 25A-B. Peptides derived from proteinase K digestion of GAS190 aligned with the full-length amino acid sequence of GAS190 (SEQ ID NO:117). FIG. 25A, individual peptides (SEQ ID NOS:932-949); FIG. 25B, schematic.

[32] FIG. 26A-B. Peptides derived from trypsin digestion of GAS190 aligned with the full-length amino acid sequence of GAS190 (SEQ ID NO:117). FIG. 26A, individual peptides (SEQ ID NOS:950-961); FIG. 26B, schematic.

[33] FIG. 27. Summary of predicted LPXTG (SEQ ID NO:931) proteins.

[34] FIG. 28-104. Topological representations of identified membrane-associated proteins. The protease cleavage sites are in red. LPXTG, SEQ ID NO:931.

[35] FIG. 105A-B. Bioinformatics-based topology predictions of all the predicted membrane proteins identified and their matching with identified peptides. Proteins are ordered by the number of predicted transmembrane domains (TMD) and, within, by their TIGR accession number. FIG. 105A shows the proteins whose peptides identified by

proteomics matched extracellular domains predicted by PSORT. FIG. 105B shows those membrane proteins whose peptides identified by proteomics matched cytoplasmic domains predicted by PSORT.

[36] FIG. 106. Comparison between found and predicted proteins for each of the four types of surface-associated proteins in *Streptococcus pyogenes* and FACS responses of those identified. LPXTG proteins: 17 proteins containing the LPXTG (SEQ ID NO:931)-anchoring motif to the cell wall were predicted to be present in the genome; 12 (71%) were found and 5 (29%) were not. Of those identified, 11 were tested and all of them were positive. Membrane proteins: 489 membrane proteins were predicted by in silico analysis; 452 (92%) were not found, whereas the number of found proteins was 37 (8%); 15 were not tested by FACS. Of those tested, 17 (77%) exhibited a positive response; 5 (23%) were negative. Lipoproteins: 11 lipoproteins out of 28 predicted by in silico analysis (39%) were found; 17 (61%) were not found. All of those identified were FACS-tested, and 9 (81%) were positive; 2 lipoproteins (19%) exhibited a negative response. Secreted proteins: 67 secreted proteins were predicted; 59 (88%) were not found, and 8 (12%) were found. Of these, one was not tested by FACS. Out of those tested, 6 (86%) were positive, and only one (14%) was negative.

[37] FIG. 107. Electron micrograph of membrane-delimited structures produced upon penicillin treatment of GAS bacteria.

[38] FIG. 108. Graph showing hyaluronic acid content of M1, M3, M6, and M23 GAS bacteria (fg/CFU).

[39] FIG. 109A-C. FACS pictograms of surface-exposed GAS antigens.

[40] FIG. 110A-C. FACS pictograms of surface-exposed GAS antigens.

[41] FIG. 111A-C. FACS pictograms of surface-exposed GAS antigens.

[42] FIG. 112. Graph showing prevalent immunoreactive antigens identified from serum samples of 6 healthy donors.

DETAILED DESCRIPTION OF THE INVENTION

[43] The invention provides compositions for preventing and/or treating *S. pyogenes* infection. These compositions comprise one or more active agents, which can be GAS antigens expressed on the surface of GAS bacteria, nucleic acid molecules encoding the GAS antigens, and/or antibodies which selectively bind to the GAS antigens.

[44] *GAS antigens*

[45] "GAS antigens" according to the invention include (1) naturally occurring immunogenic proteins of a GAS bacterium, (2) immunogenic portions of such proteins, and (3) engineered proteins or portions of proteins with amino acid sequences which retain immunogenicity and which are at least 50% identical to the amino acid sequence of a naturally occurring GAS immunogenic protein or portion thereof, such as homologs, orthologs, allelic variants, and mutants. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more). Typically, 50% identity or more between two polypeptide sequences is considered to be an indication of functional equivalence. Identity between polypeptides is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters gap open penalty = 12 and gap extension penalty = 1.

[46] Amino acid sequences for examples of GAS proteins, as well as nucleotide sequences encoding the proteins, are identified in Table 1.

[47] Preferably, a GAS antigen is shorter than a GAS protein by at least one amino acid (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 76, 80, 85, 90, 95,

100, or more amino acids). More preferably a GAS antigen lacks a transmembrane domain. Even more preferably, a GAS antigen comprises a surface-exposed domain.

[48] The invention also includes various polypeptide fragments (including immunogenic portions) of the identified GAS proteins. The length of a fragment may vary depending on the amino acid sequence of the particular GAS antigen. Typically, fragments of GAS proteins comprise at least 7 contiguous amino acids (*e.g.*, 8, 10, 12, 14, 16, 18, 20, 25, 29, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 178, 200, 203, 250 or more contiguous amino acids).

[49] Preferably the fragment comprises one or more epitopes. The fragment may comprise at least one T-cell or, preferably, a B-cell epitope of the sequence. T- and B-cell epitopes can be identified empirically (*e.g.*, using PEPSCAN (Geysen *et al.* (1984) PNAS USA 81:3998-4002; Carter (1994) Methods Mol. Biol. 36:207-223, or similar methods), or they can be predicted (*e.g.*, using the Jameson-Wolf antigenic index (Jameson, BA *et al.* 1988, CABIOS 4(1):1818-186), matrix-based approaches (Raddrizzani and Hammer (2000) Brief Bioinform. 1(2):179-189), TEPITOPE (De Lalla *et al.* (199) J. Immunol. 163:1725-1729), neural networks (Brusic *et al.* (1998) Bioinformatics 14(2):121-130), OptiMer & EpiMer (Meister *et al.* (1995) Vaccine 13(6):581-591; Roberts *et al.* (1996) AIDS Res. Hum. Retroviruses 12(7):593-610), ADEPT (Maksyutov & Zagrebelnaya (1993) Comput. Appl. Biosci. 9(3):291-297), Tsites (Feller & de la Cruz (1991) Nature 349(6311):720-721), hydrophilicity (Hopp (1993) Peptide Research 6:183-190), antigenic index (Welling *et al.* (1985)FEBS Lett. 188:215-218) or the methods disclosed in Davenport *et al.* (1995) Immunogenetics 42:392-297, etc.

[50] Other preferred fragments include (1) the N-terminal signal peptides of each identified GAS protein, (2) the identified GAS protein without its N-terminal signal peptide, (3) each identified GAS protein wherein up to 10 amino acid residues (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) are deleted from the N-terminus and/or the C-terminus, and (4) GAS polypeptides without their N-terminal amino acid residue. Some fragments

omit one or more domains of the protein (*e.g.*, omission of a signal peptide, a cytoplasmic domain, a transmembrane domain, and/or an extracellular domain).

[51] Some GAS antigens consist of immunogenic portions of GAS proteins, which can be surface exposed domains as disclosed herein. Other GAS antigens are "hybrid GAS antigens," which comprise one or more immunogenic portions of a full-length GAS protein. Hybrid GAS antigens, which also can include full-length GAS antigens, are described in detail below. Other fusion proteins can comprise, for example, one or more additional antigens and/or a tag protein, such as polyhistidine (HIS) or glutathione-S-transferase (GST).

[52] Preferably, a GAS antigen is expressed on the surface of a GAS bacterium, most preferably on the surface of more than one M type (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or 9 M types), particularly M1, M3, M6, M11, M 12, and/or M23 GAS types. GAS antigens also preferably are found on the surface of at least two different strains (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 or more strains). Preferred GAS antigens are highly conserved among multiple M types and/or multiple strains within an M type. See Table 2, which lists full-length GAS proteins and M types on which the proteins are expressed. Columns 3-13 of Table 2 list M types (*e.g.*, M1, M2, etc.). The presence these GAS proteins were detected on the surface of various strains of these M types as explained in Example 1; the number of strains tested within each type is shown in parentheses in columns 3-13. The final column lists the number of strains out of the total of 20 strains tested which express each of these GAS antigens.

[53] As indicated in Table 2, some GAS antigens are expressed on the surface of a number of different M types as well as on the surface of multiple strains within some of these M types. In some embodiments, compositions of the invention comprise one or more GAS antigens which are expressed on the surface of an M1, M3, M6, M11, M12, and/or M23 type. Preferred GAS antigens of this type GAS 5, 99, 166, 96, 103, 188, 76, 108, 142, 190, 22, 56, 77, 67, 75, 93, 18, 23, 69, 206, 249, 123, 143, 68, 25, 30, 97, 105, 187, 195,

242, 81, 101, 6, 62, 49, 63, 85, 89, 100, 179, 205, 291, 98, 104, 36, 92, 158, 178, 218, 175, 78, 131, 29, 82, 91, 165, 327, 219, 60, 86, 380, 207, 271, 74, and 685 antigens. Even more preferably, a GAS antigen is exposed on at least 10 M types (*e.g.*, GAS 5, 22, 40, 56, 67, 76, 77, 96, 99, 103, 108, 142, 166, 188, and 190).

[54] GAS antigens of the invention also include surface-exposed domains of GAS proteins 4, 5, 15, 16, 23, 24, 25, 40, 49, 54, 57, 63, 64, 68, 72, 84, 86, 87, 89, 98, 102, 103, 108, 143, 149, 152, 157, 158, 163, 166, 168, 171, 177, 188, 190, 191, 192, 193, 194, 195, 198, 201, 224, 251, 259, 262, 264, 268, 277, 282, 299, 382, 405, 406, 425, 433, 460, 469, 493, 500, 545, 558, 587, 645, 650, 685, 362-1, spy0080a, spy0272, spy0461, spy0611, spy0717, spy0792, spy1029, spy1073, spy1260, spy1613, spy1835, spy2005, spy2093, spy2178, NT01SP0246, spy0047, spy0127, and spy0686 (see Table 7).

[55] Other GAS antigens include surface-exposed domains of GAS proteins 5, 10, 23, 24, 49, 56, 63, 67, 72, 78, 81, 83, 84, 86, 89, 98, 100, 103, 157, 160, 177, 192, 194, 201, 205, 284, 286, 292, 382, 396, 405, 406, 500, spy0047 , spy0053, spy0056, spy0063, spy0069, spy0098, spy0127, spy0274, spy0611, spy0666, spy0686, spy0688, spy0731, spy0913, spy1200, spy1281, spy1721, spy1750, spy1805, spy2070, spy2092, spy2178, and gi-21909751 (see Table 8).

[56] Still others include surface-exposed domains of GAS proteins 16, 57, 68, 143, 158, 166, 171, 188, 190, 191, 192, 23, NT01SP0246, 49, 685, 63, 108, 84, 86, 89, 98, 103, 4, 149, 152, 157, 72, 405, 406, 299, 168, 251, 259, 262, 177, 264, 268, 277, 193, 194, 282, 195, 201, 40, 224, 163, 500, 198, 433, 54, 545, 469, 587, 645, 425, 493, 460, 558, 650, 5, 24, 25, 64, 87, 362-1, 382, 102, NT01SP0485, NT01SP0572, NT01SP0634, and NT01SP0877 (see Table 9).

[57] Some surface-exposed domains are shown in SEQ ID NOS:591-649. Other surface-exposed GAS antigens comprise at least 7 contiguous amino acids selected from the group consisting of SEQ ID NOS:1-281 (*i.e.*, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 50, 75, or 100 or more).

[58] GAS antigens also include the surface-exposed domains of GAS proteins 35, 54, 70, 414, 421, 425, 426, 428, 433, 434, 437, 438, 439, 457, 461, 465, 469, 472, 473, 474, 475, 477, 478, 486, 492, 494, 495, 535, 538, 540, 543, 553, 560, 561, 564, 565, 574, 576, 577, 579, 586, 587, 591, 592, 607, 609, 625, 626, 636, 640, 643, 649, 653, 657, and 663. More preferred GAS antigens comprise a surface-exposed domain of 35, 414, 437, 438, 461, 465-2, 469, 472, 473, 475, 478, 495, 538, 553, 561, 577-2, 591, 593, 636, 643, 649, or 663. Even more preferred surface-exposed GAS antigens comprise a surface-exposed domain of GAS472, GAS473, or GAS553.

[59] Other useful GAS antigens include GAS117, GAS130, GAS277, GAS236, GAS389, GAS504, GAS509, GAS366, GAS159, GAS217, GAS309, GAS372, GAS039, GAS042, GAS058, GAS290, GAS511, GAS533, GAS527, GAS294, GAS253, GAS529, GAS045, GAS095, GAS193, GAS137, GAS084, GAS384, GAS202, and GAS057 antigens, as well as M protein, GAS fibronectin-binding protein, GAS streptococcal heme-associated protein, and streptolysin S antigens.

[60] Preferred groups of GAS antigens for use in vaccines of the present invention include:

(i) GAS4, GAS24, GAS54, GAS63, GAS64, GAS72, GAS86, GAS87, GAS102, GAS149, GAS152, GAS157, GAS163, GAS168, GAS171, GAS177, GAS191, GAS192, GAS194, GAS198, GAS201, GAS224, GAS251, GAS259, GAS262, GAS264, GAS268, GAS282, GAS299, GAS382, GAS405, GAS406, GAS425, GAS433, GAS460, GAS469, GAS493, GAS500, GAS545, GAS558, GAS587, GAS645, GAS650, GAS685, GAS362-1, spy611, spy717, spy792, spy1073, NT01SP0246, and NT01SP0102;

(ii) GAS64, GAS149, GAS158, GAS166, GAS191, GAS192, GAS193, SPY1664, and SPY0861;

(iii) GAS57, GAS64, GAS72, GAS84, GAS98, GAS108, GAS152, GAS157, GAS158, GAS166, GAS191, GAS192, GAS193, GAS268, NT01SP0246, NT01SP0908 (Spy1111), and NT01SP0182 (Spy0216);

(iv) GAS64, GAS158, GAS166, GAS191, GAS192, and GAS193; and

(v) GAS5, GAS6, GAS15, GAS16p2, GAS18, GAS22, GAS23, GAS25, GAS29, GAS30, GAS36, GAS40a-RR, GAS42, GAS45, GAS49, GAS56, GAS57, GAS60, GAS62, GAS63, GAS65, GAS67, GAS68, GAS69, GAS75, GAS76, GAS77, GAS81, GAS82, GAS84, GAS85, GAS86, GAS88, GAS89, GAS91, GAS92, GAS94, GAS95, GAS96, GAS97, GAS98, M30098, GAS99, GAS100, M3_0100, GAS101, M3_0102, GAS103, M3_0104, GAS105, SPs0106, GAS108, GAS117-40+A97, GAS130, GAS137, GAS142, GAS143, M6_0157, GAS158, M6_0159, GAS159a, M6_0160, GAS165, GAS166, GAS175, GAS178, GAS179-1, GAS187, GAS188, GAS190, GAS191, GAS193, GAS195, GAS205-1, GAS206, GAS208, GAS217, GAS218, GAS218-t, GAS219-1, GAS220, GAS242, GAS249, GAS277a, GAS290, GAS294-1, GAS327, GAS380, GAS384-RR, GAS504, GAS509, GAS511, GAS527, GAS529, GAS533, GAS680, 19224134, 19224135, 19224137, and 19224141 (see Table 16).

[61] GAS 680 is annotated as a predicted CoA-binding protein and corresponds to M1 GenBank accession numbers GI:13621481 and GI:71909974, to M49 GenBank accession number GI:56808534, to M18 GenBank accession number GI:19747454, to M3 GenBank accession number GI: 28895062, and is also referred to as 'Spy0186' or 'M5005_Spy_0160' (M1), 'SpyoM01000450' (M49), 'spyM18_0185' (M18) and 'SPs0150' (M3).

[62] GAS vaccines of the invention preferably include all or a surface portion of GAS57 and/or GAS40.

GAS40 antigens

[63] GAS40 antigens are particularly useful in compositions of the invention because GAS40 proteins are highly conserved both in many M types and in multiple strains of these M types (see FIG. 1). GAS40 proteins are described in detail in WO 05/032582. See also FIG. 15. GAS40 consistently provides protection in the animal model of systemic

immunization and challenge and induction of bactericidal antibodies (see the specific Examples, below). GAS40 is an extremely highly conserved protein and appears to be exposed on the surface of most M serotypes (the only exception observed thus far is the M3 serotype).

[64] Amino acid sequences of a number of GAS40 proteins from various M strains are provided in SEQ ID NOS:17-43. The amino acid sequences of several GAS40 proteins also are contained in GenBank and have accession numbers GI:13621545 and GI:15674449 (M1); accession number GI: 21909733 (M3), and accession number GI:19745402 (M18). GAS40 proteins also are known as "Spy0269" (M1), "SpyM3_0197" (M3), "SpyM18_0256" (M18) and "prgA."

[65] A GAS40 protein typically contains a leader peptide sequence (*e.g.*, amino acids 1 – 26 of SEQ ID NO:17), a first coiled-coil region (*e.g.*, amino acids 58 – 261 of SEQ ID NO:17), a second coiled coil region (*e.g.*, amino acids 556 – 733 of SEQ ID NO:17), a leucine zipper region (*e.g.*, amino acids 673 – 701 of SEQ ID NO:17) and a transmembrane region (*e.g.*, amino acids 855 – 866 of SEQ ID NO:17).

[66] Preferred fragments of a GAS40 protein lack one or more amino acids (*e.g.*,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.*,1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of the GAS40 protein. In one embodiment, the leader sequence is removed. In another embodiment, the transmembrane region is removed. Other fragments may omit one or more other domains of the GAS40 protein.

[67] The coiled-coil regions of GAS40 are likely involved in the formation of oligomers such as dimers or trimers. Such oligomers could be homomers (containing two or more GAS40 proteins oligomerized together) or heteromers (containing one or more additional GAS proteins oligomerized with GAS40). Alternatively, two coiled-coil regions may interact together within the GAS40 protein to form oligomeric reactions between the first and second coiled-coil regions. Thus, in some embodiments the GAS40 antigen is in the

form of an oligomer. Some oligomers comprise two more GAS40 antigens. Other oligomers comprise a GAS40 antigen oligomerized to a second GAS antigen.

[68] Other useful GAS antigens include fusion proteins comprising GAS40 and GAS117. "40/117" is a GAS40 hybrid antigen in which the GAS 40 protein is placed to the N-terminus of the GAS117 protein and a HIS tag is added to the C terminus of the GAS117 protein (SEQ ID NO:234). "117/40" is a GAS40 hybrid antigen in which GAS117 is fused to GAS40 by the linker sequence YASGGGS (SEQ ID NO:278). Its amino acid sequence is shown in SEQ ID NO:233.

[69] "GAS40a-HIS" is a GAS40 antigen with a HIS tag but without the leader and hydrophobic sequences (SEQ ID NO:235). A nucleotide sequence encoding GAS40a is shown in SEQ ID NO:892 (codon 824, AGA in the wild-type sequence, was mutagenized to CGT). "GAS40aRR" is similar to GAS40a except that two additional AGA codons (334 and 335) in the coding sequence were mutated to CGT.

Hybrid GAS antigens

[70] GAS antigens can be present in compositions of the invention as individual separate polypeptides. Alternatively, at least two (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of any of the GAS antigens described above can be expressed as a single polypeptide chain, *i.e.*, a "hybrid GAS antigen." Hybrid GAS antigens offer two principal advantages. First, a polypeptide which may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner which overcomes the problem. Second, commercial manufacture is simplified because only one expression and purification produces two polypeptides, both of which are antigenically useful.

[71] A hybrid GAS antigen can comprise two or more amino acid sequences for GAS40 antigens and/or one or more other GAS antigens of the invention. Hybrids can comprise amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten or more

GAS antigens. In compositions of the invention, a GAS antigen can be present in more than one hybrid GAS antigen and/or as a non hybrid GAS antigen.

[72] A hybrid GAS antigen comprises at least two GAS antigens (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) expressed as a single polypeptide chain. Preferred hybrid GAS antigens comprise at least one surface-exposed and/or surface-associated GAS antigen. Hybrid GAS antigens offer two principal advantages. First, a polypeptide which may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner which overcomes the problem. Second, commercial manufacture is simplified because only one expression and purification produces two polypeptides, both of which are antigenically useful.

[73] Hybrid GAS antigens can be represented by the formula:

$$NH_2\text{-A-(-X-L-)}_n\text{-B-COOH}$$

[74] in which X is an amino acid sequence of a surface-exposed and/or surface-associated or secondary GAS antigen; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

[75] If an -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid antigen. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

[76] For each *n* instances of (-X-L-), linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be $NH_2\text{-}X_1\text{-}L_1\text{-}X_2\text{-}L_2\text{-COOH}$, $NH_2\text{-}X_1\text{-}X_2\text{-COOH}$, $NH_2\text{-}X_1\text{-}L_1\text{-}X_2\text{-COOH}$, $NH_2\text{-}X_1\text{-}X_2\text{-}L_2\text{-COOH}$, *etc.* Linker amino acid sequence(s) -L- will typically be short, *e.g.*,20 or fewer amino acids (*i.e.*, 20, 19, 18, 17, 16, 15, 14, 13,

12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include short peptide sequences which facilitate cloning, poly-glycine linkers (Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID NO:280), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, which aids cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker.

[77] -A- is an optional N-terminal amino acid sequence. This will typically be short, *e.g.*, 40 or fewer amino acids (*i.e.*, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking or short peptide sequences which facilitate cloning or purification (*e.g.*, *a* histidine tag His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.*, with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

[78] -B- is an optional C-terminal amino acid sequence. This will typically be short, *e.g.*, 40 or fewer amino acids (*i.e.*, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.*, His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

[79] The individual GAS antigens within the hybrid (individual -X- moieties) may be from one or more strains or from one or more M types. Where n=2, for instance, X_2 may be from the same strain or type as X_1 or from a different strain or type. Where n=3, the

strains might be (i) $X_1=X_2=X_3$, (ii) $X_1=X_2\neq X_3$, (iii) $X_1\neq X_2=X_3$, (iv) $X_1\neq X_2\neq X_3$, or (v) $X_1=X_3\neq X_2$, *etc.*

Identification of surface-exposed GAS antigens

[80] Surface-exposed and/or surface-associated GAS antigens (GAS "surfome") can be identified using any one or combination of several proteomics approaches as outlined below. These proteomics strategies have great potential for shortening the time needed for vaccine discovery when compared with other strategies, such as reverse vaccinology. Surface-exposed and/or surface-associated GAS antigens identified by these methods can be used as active agents in compositions for preventing and for treating *S. pyogenes* infections.

[81] One embodiment is described in Example 13. Briefly, the surface of whole GAS bacterial cells is digested in vivo under physiological conditions using reagents which cleave proteins. Typically the reagents are proteases (*e.g.*, trypsin, protease K, papain), although any protein cleavage reagent can be used. These reagents include, for example, formic acid, hydroxylamine, BNPS-skatole (3-bromo-3-methyl-2-(o-nitrophenyl-sulfenyl)- indolenine), which cleaves at Trp residues), cyanogen bromide (which cleaves polypeptides on the carboxyl side of methionine residues), metal chelate reagents such as Fe-EDTA, and the like. Proteases can be either free or anchored, this latter condition favouring the identification of surface extruding regions. Combinations of more than one protein cleavage reagent can be used. The recovered peptides are then separated by liquid chromatography and identified by tandem mass spectrometry. The actual accessibility of identified proteins to the immune system can be assessed by fluorescence-activated cell sorting (FACS) analysis. This proteomic approach permits validation of software-based topology predictions and vice versa.

[82] Another embodiment is described in Example 14. This approach involves overproduction of membrane-delimited from GAS bacteria after antibiotic treatment. See Hakenbeck *et al.*, J. Bacteriol. 155, 1372-81, 1983, which is incorporated herein by

reference. Either wild-type GAS bacteria or mutant GAS bacteria, for example those with "leaky" or destabilized peptidoglycan cell walls, can be used in this method. GAS bacteria naturally produce membrane-delimited structures which are released into the growth medium. When the bacteria are treated with an antibiotic that interferes with the synthesis of the cell-wall such as penicillins, cephalosporins, glycopeptides and cycloserine, production of these membrane-delimited structures increases. Vancomycin, a glycopeptide which inhibits both cell wall synthesis and the sortase, interferes with surface protein anchoring which is catalyzed by sortases and can be used to further increase the overproduction of membrane-delimited structures. The membrane-delimited structures contain GAS proteins which are potential vaccine candidates. The GAS proteins can be separated by electrophoresis and identified using mass spectrometry (*e.g.*, MALDI-TOF). Alternatively, the GAS proteins can be digested with proteases, and the resulting fragments can be separated by liquid chromatography and identified using tandem mass spectrometry.

[83] A third embodiment is described in Example 15. In this method, cell wall and/or membrane fractions are generated by chemical cell fractionation of bacterial cells using, for example, 6 M guanidinium, urea, or SDS. The cell wall is insoluble in these reagents. This property allows the isolation of the cell wall and identification of anchored cell wall proteins. GAS proteins in these fractions can be separated and identified as described above.

[84] A fourth embodiment involves labeling cell surface GAS proteins (*e.g.*, by biotinylation), lysing the cells, and isolating labeled proteins using affinity chromatography. The isolated proteins can be separated by electrophoresis and identified using mass spectrometry. Alternatively, the isolated proteins can be digested in solution, followed by isolation of labeled peptides by affinity chromatography, separation of the labeled peptides by liquid chromatography, and identification of the labeled peptides using tandem mass spectrometry. These methods selectively isolate the labeled peptides, therefore they allow identification of the truly exposed domains. In this case, the use of

two affinity chromatography steps results in a reduction of complexity of the sample to be loaded on the chromatography column.

[85] For all the above embodiments a mutant can be used which harbors a deleted gene for one of the more abundant known surface-exposed antigens, such as M protein and C5a peptidase. These mutants will increase the probability of spotting previously unidentified, less abundant surface proteins.

[86] Analysis of the bacterial surfome provides powerful methods of identifying antigens useful in vaccines against *S. pyogenes*. For example, using these techniques, as described below, we identified a protein, Spy0416 (GAS57), which confers a remarkable protection in mice against the highly virulent M3 (MGAS315) strain. Spy0416 is a 1647 amino acid protein, carrying a C-terminal LPXTG-like motif, which shares 48% similarity with the C5a peptidase precursor. See SEQ ID NO:118. The protein has a Ca-dependent serine protease activity (Fernandez-Espla, App. Env. Microbiol., 2000) which maps within the first 600 amino acids of the protein. Spy0416 has a homolog in Group B Streptococcus (GBS) (cspA) which was proposed to be involved in GBS virulence by potentially protecting the bacterium from opsonophagocytic killing (Harris *et al.*, J. Clin. Invest. 111, 61-70, 2003). Lei and co-workers recently found that a 31 kDa N-terminal fragment of Spy0416 is released in the supernatant of GAS cultures and that the protein is well recognized by sera from GAS-infected patients (Lei *et al.*, Inf. Immunol. 68, 6807-18, 2000). Based on the 5 available *Streptococcus pyogenes* genome sequences, the protein appears to be highly conserved (over 98%) and preliminary data on surface expression on a panel of 20 different GAS strains indicates that Spy0416 is a major component of over 70% of the circulating strains. It is, therefore, a preferred antigen for use in immunogenic compositions, either alone or in combination with one or more other GAS antigens.

Nucleic acid molecules

[87] The sequence listing provides coding sequences for the surface-exposed and/or surface-associated domains disclosed herein and their full-length proteins, as well as for the

additional disclosed secondary GAS antigens. Any nucleotide sequence which encodes a particular antigen, however, can be used in a compositions of the invention, for example as a DNA vaccine, or to produce a GAS antigen recombinantly, as described below. The full genomic sequences of at least three GAS strains are publicly available and can be used to obtain coding sequences for GAS antigens. The genomic sequence of an M1 GAS strain is reported in Ferretti *et al.*, Proc. Natl. Acad. Sci. U.S.A. 98, 4658-63, 2002. The genomic sequence of an M3 GAS strain is reported in Beres *et al.*, Proc. Natl. Acad. Sci. U.S.A. 99, 10078 – 83, 2002. The genomic sequence of an M18 GAS strain is reported in Smooet *et al.*, Proc. Natl. Acad. Sci. U.S.A. 99, 4668 – 73, 2002.

[88] The invention includes nucleic acid molecules which encode the identified GAS proteins and protein fragments. The invention also includes nucleic acid molecules comprising nucleotide sequences having at least 50% sequence identity to such molecules. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more). Identity between nucleotide sequences is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters gap open penalty = 12 and gap extension penalty = 1.

[89] The invention also provides nucleic acid molecules which can hybridize to these molecules. Hybridization reactions can be performed under conditions of different "stringency." Conditions which increase stringency of a hybridization reaction are widely known and published in the art. See, *e.g.*, page 7.52 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 1989. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25oC, 37 oC, 50 oC, 55 oC, and 68 oC; buffer concentrations of 10X SSC, 6X SSC, 1X SSC, and 0.1X SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of

1, 2, or 15 minutes; and wash solutions of 6X SSC, 1X SSC, 0.1X SSC, or de-ionized water. Hybridization techniques and their optimization are well known in the art. See, *e.g.*, Sambrook, 1989; Ausubel *et al.*, eds., Short Protocols in Molecular Biology, 4th ed., 1999; U.S. Patent 5,707,829; Ausubel *et al.*, eds., Current Protocols in Molecular Biology, Supplement 30, 1987.

[90] In some embodiments, nucleic acid molecules of the invention hybridize to a target under low stringency conditions; in other embodiments, nucleic acid molecules of the invention hybridize under intermediate stringency conditions; in preferred embodiments, nucleic acid molecules of the invention hybridize under high stringency conditions. An example of a low stringency hybridization condition is 50oC and 10X SSC. An example of an intermediate stringency hybridization condition is 55oC and 1X SSC. An example of a high stringency hybridization condition is 68oC and 0.1X SSC.

[91] Nucleic acid molecules comprising fragments of these sequences are also included in the invention. These comprise at least n consecutive nucleotides of these sequences and, depending on the particular sequence, n is 10 or more (*e.g.*, 12, 14, 15, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more).

[92] Nucleic acids (and polypeptides) of the invention may include sequences which:

(a) are identical (*i.e.*, 100% identical) to the sequences disclosed in the sequence listing;

(b) share sequence identity with the sequences disclosed in the sequence listing;

(c) have 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 single nucleotide or amino acid alterations (deletions, insertions, substitutions), which may be at separate locations or may be contiguous, as compared to the sequences of (a) or (b); and,

d) when aligned with a particular sequence from the sequence listing using a pairwise alignment algorithm, a moving window of x monomers (amino acids or

nucleotides) moving from start (N-terminus or 5′) to end (C-terminus or 3′), such that for an alignment that extends to p monomers (where p>x) there are p-x+1 such windows, each window has at least x·y identical aligned monomers, where: x is selected from 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200; y is selected from 0.50, 0.60, 0.70, 0.75, 0.80, 0.85, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99; and if x·y is not an integer then it is rounded up to the nearest integer. The preferred pairwise alignment algorithm is the Needleman-Wunsch global alignment algorithm [Needleman & Wunsch (1970) J. Mol. Biol. 48, 443-453], using default parameters (*e.g.*, with Gap opening penalty = 10.0, and with Gap extension penalty = 0.5, using the EBLOSUM62 scoring matrix). This algorithm is conveniently implemented in the needle tool in the EMBOSS package [Rice *et al.* (2000) Trends Genet. 16:276-277].

[93] The nucleic acids and polypeptides of the invention may additionally have further sequences to the N-terminus/5′ and/or C-terminus/3′ of these sequences (a) to (d).

Antibodies

[94] Antibodies can be generated to bind specifically to a surface-exposed and/or surface-associated GAS antigen or to a secondary GAS or non-GAS polypeptide antigen disclosed herein. The term "antibody" includes intact immunoglobulin molecules, as well as fragments thereof which are capable of binding an antigen. These include hybrid (chimeric) antibody molecules (*e.g.*, Winter *et al.*, Nature 349, 293-99, 1991; U.S. Patent 4,816,567); F(ab′)2 and F(ab) fragments and Fv molecules; non-covalent heterodimers (*e.g.*, Inbar *et al.*, Proc. Natl. Acad. Sci. U.S.A. 69, 2659-62, 1972; Ehrlich *et al.*, Biochem 19, 4091-96, 1980); single-chain Fv molecules (sFv) (*e.g.*, Huston *et al.*, Proc. Natl. Acad. Sci. U.S.A. 85, 5897-83, 1988); dimeric and trimeric antibody fragment constructs; minibodies (*e.g.*, Pack *et al.*, Biochem 31, 1579-84, 1992; Cumber *et al.*, J. Immunology 149B, 120-26, 1992); humanized antibody molecules (*e.g.*, Riechmann *et al.*, Nature 332, 323-27, 1988; Verhoeyan *et al.*, Science 239, 1534-36, 1988; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994); and any functional

fragments obtained from such molecules, as well as antibodies obtained through non-conventional processes such as phage display. Preferably, the antibodies are monoclonal antibodies. Methods of obtaining monoclonal antibodies are well known in the art.

[95] Typically, at least 6, 7, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids. Various immunoassays (*e.g.*, Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art) can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen. A preparation of antibodies which specifically bind to a particular antigen typically provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, the antibodies do not detect other proteins in immunochemical assays and can immunoprecipitate the particular antigen from solution.

Generation of antibodies

[96] GAS antigens or non-GAS polypeptide antigens (described below) can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, an antigen can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially useful.

[97] Monoclonal antibodies which specifically bind to an antigen can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B cell hybridoma technique, and the EBV hybridoma technique (Kohler *et al.*, Nature 256, 495 497, 1985; Kozbor *et al.*, J. Immunol. Methods 81, 31 42, 1985; Cote *et al.*, Proc. Natl. Acad. Sci. 80, 2026 2030, 1983; Cole *et al.*, Mol. Cell Biol. 62, 109 120, 1984).

[98] In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, Proc. Natl. Acad. Sci. 81, 6851 6855, 1984; Neuberger *et al.*, Nature 312, 604 608, 1984; Takeda *et al.*, Nature 314, 452 454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions.

[99] Alternatively, humanized antibodies can be produced using recombinant methods, as described below. Antibodies which specifically bind to a particular antigen can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

[100] Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to a particular antigen. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random

combinatorial immunoglobin libraries (Burton, Proc. Natl. Acad. Sci. 88, 11120 23, 1991).

[101] Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, Eur. J. Cancer Prev. 5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, Nat. Biotechnol. 15, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, J. Biol. Chem. 269, 199-206.

[102] A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, Int. J. Cancer 61, 497-501; Nicholls *et al.*, 1993, J. Immunol. Meth. 165, 81-91).

[103] Antibodies which specifically bind to a particular antigen also can be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, Proc. Natl. Acad. Sci. 86, 3833 3837, 1989; Winter *et al.*, Nature 349, 293 299, 1991).

[104] Chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

[105] Antibodies can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which the relevant antigen is bound.

The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Production of polypeptide antigens

Recombinant production of polypeptides

[106] Any nucleotide sequence which encodes a particular antigen can be used to produce that antigen recombinantly. If desired, an antibody can be produced recombinantly once its amino acid sequence is known.

[107] Examples of sequences which can be used to produce GAS antigens of the invention are identified in Table 1. Nucleic acid molecules encoding surface-exposed and/or surface-associated or secondary GAS antigens can be isolated from the appropriate *S. pyogenes* bacterium using standard nucleic acid purification techniques or can be synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating nucleic acids are routine and are known in the art. Any such technique for obtaining nucleic acid molecules can be used to obtain a nucleic acid molecule which encodes a particular antigen. Sequences encoding a particular antigen or antibody can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, Nucl. Acids Res. Symp. Ser. 215 223, 1980; Horn *et al.* Nucl. Acids Res. Symp. Ser. 225 232, 1980).

[108] cDNA molecules can be made with standard molecular biology techniques, using mRNA as a template. cDNA molecules can thereafter be replicated using molecular biology techniques well known in the art. An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either genomic DNA or cDNA as a template.

[109] If desired, nucleotide sequences can be engineered using methods generally known in the art to alter antigen-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide

or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

[110] Sequence modifications, such as the addition of a purification tag sequence or codon optimization, can be used to facilitate expression. For example, the N-terminal leader sequence may be replaced with a sequence encoding for a tag protein such as polyhistidine ("HIS") or glutathione S-transferase ("GST"). Such tag proteins may be used to facilitate purification, detection, and stability of the expressed protein. Codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half life which is longer than that of a transcript generated from the naturally occurring sequence. These methods are well known in the art and are further described in WO05/032582.

Expression vectors

[111] A nucleic acid molecule which encodes an antigen or antibody can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing coding sequences and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination.

Host cells

[112] The heterologous host can be prokaryotic or eukaryotic. *E. coli* is a preferred host cell, but other suitable hosts include *Lactococcus lactis, Lactococcus cremoris, Bacillus*

subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria (*e.g., M. tuberculosis*), yeasts, etc.

[113] A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post translational activities are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of a foreign protein. See WO 01/98340.

[114] Expression constructs can be introduced into host cells using well-established techniques which include, but are not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun" methods, and DEAE- or calcium phosphate-mediated transfection.

[115] Host cells transformed with expression vectors can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell can be secreted or contained intracellularly depending on the nucleotide sequence and/or the expression vector used. Those of skill in the art understand that expression vectors can be designed to contain signal sequences which direct secretion of soluble antigens through a prokaryotic or eukaryotic cell membrane.

Purification

[116] Antigens used in the invention can be isolated from the appropriate *Streptococcus pyogenes* bacterium or from a host cell engineered to produce GAS or non-GAS antigens. A purified polypeptide antigen is separated from other components in the cell, such as proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified polypeptide antigens is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis. Where appropriate, polypeptide antigens can be solubilized, for example, with urea.

Chemical synthesis

[117] GAS antigens, as well as other antigens used in compositions of the invention, can be synthesized, for example, using solid phase techniques. See, *e.g.*, Merrifield, J. Am. Chem. Soc. 85, 2149 54, 1963; Roberge *et al.*, Science 269, 202 04, 1995. Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of a surface-exposed and/or surface-associated GAS antigen can be separately synthesized and combined using chemical methods to produce a full-length molecule.

[118] Nucleic acid molecules which encode antibodies or polypeptide antigens can be synthesized by conventional methodology, such as the phosphate triester method (Hunkapiller, M. *et al.* (1984), Nature 310: 105-111) or by the chemical synthesis of nucleic acids (Grantham, R. *et al.* (1981), Nucleic Acids Res. 9: r43-r74).

Immunogenic, Diagnostic, and Therapeutic Compositions

[119] The invention also provides compositions for use as medicaments (*e.g.*, as immunogenic compositions or vaccines) or as diagnostic reagents for detecting a GAS infection in a host subject. It also provides the use of the compositions in the manufacture of: (i) a medicament for treating or preventing infection due to GAS bacteria; (ii) a diagnostic reagent for detecting the presence of GAS bacteria or of antibodies raised against GAS bacteria; and/or (iii) a reagent which can raise antibodies against GAS bacteria.

[120] For example, GAS antigens or nucleic acids encoding the antigens can be used in the manufacture of a diagnostic reagent for detecting the presence of a GAS infection or for detecting antibodies raised against GAS bacteria, or in the manufacture of a reagent which can raise antibodies against GAS bacteria. Nucleic acids encoding GAS antigens can be detected by contacting a nucleic acid probe with a biological sample under hybridizing conditions to form duplexes and detecting the duplexes as is known in the art. A GAS antigen can be detected using antibodies which specifically bind to the GAS antigen. Similarly, antibodies to GAS antigens can be used to detect GAS antigens by contacting a biological sample under conditions suitable for the formation of antibody-antigen complexes and detecting any complexes formed. The invention also provides kits comprising reagents suitable for use these methods.

Therapeutic compositions

[121] Compositions of the invention are useful for preventing and/or treating *S. pyogenes* infection. Compositions containing GAS antigens are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of such compositions preferably is between 6 and 8, preferably about 7. The pH can be maintained by the use of a buffer. The composition can be sterile and/or pyrogen free. The composition can be isotonic with respect to humans.

[122] Vaccines according to the invention may be used either prophylactically or therapeutically, but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of a *Streptococcus pyogenes* infection. The animal is preferably a mammal, most preferably a human. The methods involve administering to the animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention.

[123] Some compositions of the invention comprise at least two surface-exposed GAS antigens as described above. Other compositions of the invention comprise at least one nucleic acid molecule which encodes two surface-exposed GAS antigens. Still other compositions of the invention comprise at least two antibodies, each of which specifically binds to one of two surface-exposed GAS antigens. Preferred compositions of the invention comprise at least one of the surface-exposed GAS antigens is a GAS40 antigen and the other antigen is any other GAS antigen; at least one nucleic acid molecule encoding the two antigens, or at least two antibodies which specifically bind to the two antigens. Some compositions comprise one or more additional GAS antigens, a nucleic acid molecule encoding the additional antigen(s), or an antibody which specifically binds to the additional antigen(s); of these antigens, GAS117 is preferred.

[124] As described above, some compositions of the invention comprise a nucleic acid molecule which encodes the at least two GAS antigens and, optionally, other antigens which can be included in the composition (see below). See, *e.g.*, Robinson & Torres (1997) Seminars in Immunology 9:271-283; Donnelly *et al.* (1997) Ann. Rev Immunol 15:617-648; Scott-Taylor & Dalgleish (2000) Expert Opin Investig Drugs 9:471-480; Apostolopoulos & Plebanski (2000) Curr Opin Mol Ther 2:441-447; Ilan (1999) Curr Opin Mol Ther 1:116-120; Dubensky *et al.* (2000) Mol Med 6:723-732; Robinson & Pertmer (2000) Adv Virus Res 55:1-74; Donnelly *et al.* (2000) Am J Respir Crit Care Med 162(4 Pt 2):S190-193; Davis (1999) Mt. Sinai J. Med. 66:84-90. Typically the nucleic acid molecule is a DNA molecule, *e.g.*, in the form of a plasmid.

[125] Compositions for treating *S. pyogenes* infections comprise at least one antibody which specifically binds to a GAS antigen and, optionally, an antibody which specifically binds to a non-GAS antigen. Some compositions of the invention are immunogenic and comprise one or more polypeptide antigens, while other immunogenic compositions comprise nucleic acid molecules which encode a surface-exposed and/or surface-associated GAS antigen and, optionally, a secondary GAS antigen or a non-GAS antigen. See, *e.g.*, Robinson & Torres (1997) Seminars in Immunology 9:271-283; Donnelly *et al.* (1997) Ann. Rev Immunol 15:617-648; Scott-Taylor & Dalgleish (2000) Expert Opin Investig Drugs 9:471-480; Apostolopoulos & Plebanski (2000) Curr Opin Mol Ther 2:441-447; Ilan (1999) Curr Opin Mol Ther 1:116-120; Dubensky *et al.* (2000) Mol Med 6:723-732; Robinson & Pertmer (2000) Adv Virus Res 55:1-74; Donnelly *et al.* (2000) Am J Respir Crit Care Med 162(4 Pt 2):S190-193Davis (1999) Mt. Sinai J. Med. 66:84-90. Typically the nucleic acid molecule is a DNA molecule, *e.g.*, in the form of a plasmid.

[126] Other compositions of the invention comprise at least one active agent. Compositions for preventing *S. pyogenes* infection can comprise as an active agent either a polypeptide comprising a GAS antigen of the invention or a nucleic acid molecule which encodes the polypeptide.

[127] In some embodiments, compositions of the invention can include one or more additional active agents. Such agents include, but are not limited to, (a) another GAS antigen of the invention, preferably a surface-exposed antigen, (b) a polypeptide antigen which is useful in a pediatric vaccine, (c) a polypeptide antigen which is useful in a vaccine for elderly or immunocompromised individuals, (d) a nucleic acid molecule encoding (a)-(c), and an antibody which specifically binds to (a)-(c).

Additional antigens

[128] Compositions of the invention may be administered in conjunction with one or more antigens for use in therapeutic, prophylactic, or diagnostic methods of the present

invention. Preferred antigens include those listed below. Additionally, the compositions of the present invention may be used to treat or prevent infections caused by any of the below-listed pathogens. In addition to combination with the antigens described below, the compositions of the invention may also be combined with an adjuvant as described herein.

[129] Antigens for use with the invention include, but are not limited to, one or more of the following antigens set forth below, or antigens derived from one or more of the pathogens set forth below:

A. Bacterial Antigens

[130] Bacterial antigens suitable for use in the invention include proteins, polysaccharides, lipopolysaccharides, and outer membrane vesicles which may be isolated, purified or derived from a bacteria. In addition, bacterial antigens may include bacterial lysates and inactivated bacteria formulations. Bacteria antigens may be produced by recombinant expression. Bacterial antigens preferably include epitopes which are exposed on the surface of the bacteria during at least one stage of its life cycle. Bacterial antigens are preferably conserved across multiple serotypes. Bacterial antigens include antigens derived from one or more of the bacteria set forth below as well as the specific antigens examples identified below.

[131] *Neisseria meningitides*: *Meningitides* antigens may include proteins (such as those identified in References 1 – 7), saccharides (including a polysaccharide, oligosaccharide or lipopolysaccharide), or outer-membrane vesicles (References 8, 9, 10, 11) purified or derived from *N. meningitides* serogroup such as A, C, W135, Y, and/or B. *Meningitides* protein antigens may be selected from adhesions, autotransporters, toxins, Fe acquisition proteins, and membrane associated proteins (preferably integral outer membrane protein).

[132] *Streptococcus pneumoniae*: *Streptococcus pneumoniae* antigens may include a saccharide (including a polysaccharide or an oligosaccharide) and/or protein from *Streptococcus*

pneumoniae. Saccharide antigens may be selected from serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. Protein antigens may be selected from a protein identified in WO 98/18931, WO 98/18930, US Patent No. 6,699,703, US Patent No. 6,800,744, WO 97/43303, and WO 97/37026. *Streptococcus pneumoniae* proteins may be selected from the Poly Histidine Triad family (PhtX), the Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpX truncate-LytX truncate chimeric proteins, pneumolysin (Ply), PspA, PsaA, Sp128, Sp101, Sp130, Sp125 or Sp133.

[133] *Streptococcus pyogenes* (Group A Streptococcus): Group A *Streptococcus* antigens may include a protein identified in WO 02/34771 or WO 2005/032582 (including GAS 40), fusions of fragments of GAS M proteins (including those described in WO 02/094851, and Dale, Vaccine (1999) 17:193-200, and Dale, Vaccine 14(10): 944-948), fibronectin binding protein (Sfb1), Streptococcal heme-associated protein (Shp), and Streptolysin S (SagA).

[134] *Moraxella catarrhalis*: *Moraxella* antigens include antigens identified in WO 02/18595 and WO 99/58562, outer membrane protein antigens (HMW-OMP), C-antigen, and/or LPS.

[135] *Bordetella pertussis*: Pertussis antigens include petussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B. pertussis*, optionally also combination with pertactin and/or agglutinogens 2 and 3 antigen.

[136] *Staphylococcus aureus*: *Staphylococcus aureus* antigens include *S. aureus* type 5 and 8 capsular polysaccharides optionally conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A, such as StaphVAX™, or antigens derived from surface proteins, invasins (leukocidin, kinases, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule, Protein A), carotenoids, catalase production, Protein A, coagulase, clotting factor, and/or membrane-damaging toxins (optionally detoxified) that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin).

[137] *Staphylococcus epidermis*: *S. epidermidis* antigens include slime-associated antigen (SAA).

[138] *Clostridium tetani* (Tetanus): Tetanus antigens include tetanus toxoid (TT), preferably used as a carrier protein in conjunction/conjugated with the compositions of the present invention.

[139] *Cornynebacterium diphtheriae* (Diphtheria): Diphtheria antigens include diphtheria toxin, preferably detoxified, such as CRM197. Additionally antigens capable of modulating, inhibiting or associated with ADP ribosylation are contemplated for combination/co-administration/conjugation with the compositions of the present invention. The diphtheria toxoids may be used as carrier proteins.

[140] *Haemophilus influenzae B* (Hib): Hib antigens include a Hib saccharide antigen.

[141] *Pseudomonas aeruginosa*: *Pseudomonas* antigens include endotoxin A, Wzz protein, P. aeruginosa LPS, more particularly LPS isolated from PAO1 (O5 serotype), and/or Outer Membrane Proteins, including Outer Membrane Proteins F (OprF) (Infect Immun. 2001 May; 69(5): 3510-3515).

[142] *Legionella pneumophila.* Bacterial antigens may be derived from *Legionella pneumophila.*

[143] *Streptococcus agalactiae* (Group B Streptococcus): Group B *Streptococcus* antigens include a protein or saccharide antigen identified in WO 02/34771, WO 03/093306, WO 04/041157, or WO 2005/002619 (including proteins GBS 80, GBS 104, GBS 276 and GBS 322, and including saccharide antigens derived from serotypes Ia, Ib, Ia/c, II, III, IV, V, VI, VII and VIII).

[144] *Neiserria gonorrhoeae*: *Gonorrhoeae* antigens include Por (or porin) protein, such as PorB (see Zhu et al., Vaccine (2004) 22:660 – 669), a transferring binding protein, such as TbpA and TbpB (See Price et al., Infection and Immunity (2004) 71(1):277 – 283), a

opacity protein (such as Opa), a reduction-modifiable protein (Rmp), and outer membrane vesicle (OMV) preparations (see Plante et al., J Infectious Disease (2000) 182:848 – 855), also see e.g. WO99/24578, WO99/36544, WO99/57280, WO02/079243).

[145] *Chlamydia trachomatis*: *Chlamydia trachomatis* antigens include antigens derived from serotypes A, B, Ba and C (agents of trachoma, a cause of blindness), serotypes L1, L2 & L3 (associated with *Lymphogranuloma venereum*), and serotypes, D-K. Chlamydia trachomas antigens may also include an antigen identified in WO 00/37494, WO 03/049762, WO 03/068811, or WO 05/002619, including PepA (CT045), LcrE (CT089), ArtJ (CT381), DnaK (CT396), CT398, OmpH-like (CT242), L7/L12 (CT316), OmcA (CT444), AtosS (CT467), CT547, Eno (CT587), HrtA (CT823), and MurG (CT761).

[146] *Treponema pallidum* (Syphilis): Syphilis antigens include TmpA antigen.

[147] *Haemophilus ducreyi* (causing chancroid): Ducreyi antigens include outer membrane protein (DsrA).

[148] *Enterococcus faecalis* or *Enterococcus faecium*: Antigens include a trisaccharide repeat or other *Enterococcus* derived antigens provided in US Patent No. 6,756,361.

[149] *Helicobacter pylori*: *H. pylori* antigens include Cag, Vac, Nap, HopX, HopY and/or urease antigen.

[150] *Staphylococcus saprophyticus*: Antigens include the 160 kDa hemagglutinin of *S. saprophyticus* antigen.

[151] *Yersinia enterocolitica* antigens include LPS (Infect Immun. 2002 August; 70(8): 4414).

[152] *E. coli*: *E. coli* antigens may be derived from enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffusely adhering *E. coli* (DAEC), enteropathogenic *E. coli* (EPEC), and/or enterohemorrhagic *E. coli* (EHEC).

[153] *Bacillus anthracis* (anthrax): *B. anthracis* antigens are optionally detoxified and may be selected from A-components (lethal factor (LF) and edema factor (EF)), both of which can share a common B-component known as protective antigen (PA).

[154] *Yersinia pestis* (plague): Plague antigens include F1 capsular antigen (Infect Immun. 2003 Jan; 71(1)): 374-383, LPS (Infect Immun. 1999 Oct; 67(10): 5395), *Yersinia pestis* V antigen (Infect Immun. 1997 Nov; 65(11): 4476-4482).

[155] *Mycobacterium tuberculosis*: Tuberculosis antigens include lipoproteins, LPS, BCG antigens, a fusion protein of antigen 85B (Ag85B) and/or ESAT-6 optionally formulated in cationic lipid vesicles (Infect Immun. 2004 October; 72(10): 6148), *Mycobacterium tuberculosis* (Mtb) isocitrate dehydrogenase associated antigens (Proc Natl Acad Sci U S A. 2004 Aug 24; 101(34): 12652), and/or MPT51 antigens (Infect Immun. 2004 July; 72(7): 3829).

[156] *Rickettsia*: Antigens include outer membrane proteins, including the outer membrane protein A and/or B (OmpB) (Biochim Biophys Acta. 2004 Nov 1;1702(2):145), LPS, and surface protein antigen (SPA) (J Autoimmun. 1989 Jun;2 Suppl:81).

[157] *Listeria monocytogenes*. Bacterial antigens may be derived from Listeria monocytogenes.

[158] *Chlamydia pneumoniae*: Antigens include those identified in WO 02/02606.

[159] *Vibrio cholerae*: Antigens include proteinase antigens, LPS, particularly lipopolysaccharides of *Vibrio cholerae* II, O1 Inaba O-specific polysaccharides, V. cholera O139, antigens of IEM108 vaccine (Infect Immun. 2003 Oct;71(10):5498-504), and/or Zonula occludens toxin (Zot).

[160] *Salmonella typhi* (typhoid fever): Antigens include capsular polysaccharides preferably conjugates (Vi, i.e. vax-TyVi).

[161] *Borrelia burgdorferi* (Lyme disease): Antigens include lipoproteins (such as OspA, OspB, Osp C and Osp D), other surface proteins such as OspE-related proteins (Erps), decorin-binding proteins (such as DbpA), and antigenically variable VI proteins. , such as antigens associated with P39 and P13 (an integral membrane protein, Infect Immun. 2001 May; 69(5): 3323-3334), VlsE Antigenic Variation Protein (J Clin Microbiol. 1999 Dec; 37(12): 3997).

[162] *Porphyromonas gingivalis*: Antigens include *P. gingivalis* outer membrane protein (OMP).

[163] *Klebsiella*: Antigens include an OMP, including OMP A, or a polysaccharide optionally conjugated to tetanus toxoid.

[164] Further bacterial antigens of the invention may be capsular antigens, polysaccharide antigens or protein antigens of any of the above. Further bacterial antigens may also include an outer membrane vesicle (OMV) preparation. Additionally, antigens include live, attenuated, and/or purified versions of any of the aforementioned bacteria. The antigens of the present invention may be derived from gram-negative or gram-positive bacteria. The antigens of the present invention may be derived from aerobic or anaerobic bacteria.

[165] Additionally, any of the above bacterial-derived saccharides (polysaccharides, LPS, LOS or oligosaccharides) can be conjugated to another agent or antigen, such as a carrier protein (for example CRM197). Such conjugation may be direct conjugation effected by reductive amination of carbonyl moieties on the saccharide to amino groups on the protein, as provided in US Patent No. 5,360,897 and Can J Biochem Cell Biol. 1984 May;62(5):270-5. Alternatively, the saccharides can be conjugated through a linker, such as, with succinamide or other linkages provided in Bioconjugate Techniques, 1996 and CRC, Chemistry of Protein Conjugation and Cross-Linking, 1993.

B. Viral Antigens

[166] Viral antigens suitable for use in the invention include inactivated (or killed) virus, attenuated virus, split virus formulations, purified subunit formulations, viral proteins which may be isolated, purified or derived from a virus, and Virus Like Particles (VLPs). Viral antigens may be derived from viruses propagated on cell culture or other substrate. Alternatively, viral antigens may be expressed recombinantly. Viral antigens preferably include epitopes which are exposed on the surface of the virus during at least one stage of its life cycle. Viral antigens are preferably conserved across multiple serotypes or isolates. Viral antigens include antigens derived from one or more of the viruses set forth below as well as the specific antigens examples identified below.

[167] *Orthomyxovirus*: Viral antigens may be derived from an *Orthomyxovirus*, such as Influenza A, B and C. *Orthomyxovirus* antigens may be selected from one or more of the viral proteins, including hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M1), membrane protein (M2), one or more of the transcriptase components (PB1, PB2 and PA). Preferred antigens include HA and NA.

[168] Influenza antigens may be derived from interpandemic (annual) flu strains. Alternatively influenza antigens may be derived from strains with the potential to cause pandemic a pandemic outbreak (i.e., influenza strains with new haemagglutinin compared to the haemagglutinin in currently circulating strains, or influenza strains which are pathogenic in avian subjects and have the potential to be transmitted horizontally in the human population, or influenza strains which are pathogenic to humans).

[169] Paramyxoviridae viruses: Viral antigens may be derived from Paramyxoviridae viruses, such as Pneumoviruses (RSV), Paramyxoviruses (PIV) and Morbilliviruses (Measles).

[170] Pneumovirus: Viral antigens may be derived from a Pneumovirus, such as Respiratory syncytial virus (RSV), Bovine respiratory syncytial virus, Pneumonia virus of mice, and Turkey rhinotracheitis virus. Preferably, the Pneumovirus is RSV. Pneumovirus

antigens may be selected from one or more of the following proteins, including surface proteins Fusion (F), Glycoprotein (G) and Small Hydrophobic protein (SH), matrix proteins M and M2, nucleocapsid proteins N, P and L and nonstructural proteins NS1 and NS2. Preferred Pneumovirus antigens include F, G and M. See e.g., J Gen Virol. 2004 Nov; 85(Pt 11):3229). Pneumovirus antigens may also be formulated in or derived from chimeric viruses. For example, chimeric RSV/PIV viruses may comprise components of both RSV and PIV.

[171] Paramyxovirus: Viral antigens may be derived from a Paramyxovirus, such as Parainfluenza virus types 1 – 4 (PIV), Mumps, Sendai viruses, Simian virus 5, Bovine parainfluenza virus and Newcastle disease virus. Preferably, the Paramyxovirus is PIV or Mumps. Paramyxovirus antigens may be selected from one or more of the following proteins: Hemagglutinin –Neuraminidase (HN), Fusion proteins F1 and F2, Nucleoprotein (NP), Phosphoprotein (P), Large protein (L), and Matrix protein (M). Preferred Paramyxovirus proteins include HN, F1 and F2. Paramyxovirus antigens may also be formulated in or derived from chimeric viruses. For example, chimeric RSV/PIV viruses may comprise components of both RSV and PIV. Commercially available mumps vaccines include live attenuated mumps virus, in either a monovalent form or in combination with measles and rubella vaccines (MMR).

[172] Morbillivirus: Viral antigens may be derived from a Morbillivirus, such as Measles. Morbillivirus antigens may be selected from one or more of the following proteins: hemagglutinin (H), Glycoprotein (G), Fusion factor (F), Large protein (L), Nucleoprotein (NP), Polymerase phosphoprotein (P), and Matrix (M). Commercially available measles vaccines include live attenuated measles virus, typically in combination with mumps and rubella (MMR).

[173] Picornavirus: Viral antigens may be derived from Picornaviruses, such as Enteroviruses, Rhinoviruses, Heparnavirus, Cardioviruses and Aphthoviruses. Antigens derived from Enteroviruses, such as Poliovirus are preferred.

[174] Enterovirus: Viral antigens may be derived from an Enterovirus, such as Poliovirus types 1, 2 or 3, Coxsackie A virus types 1 to 22 and 24, Coxsackie B virus types 1 to 6, Echovirus (ECHO) virus) types 1 to 9, 11 to 27 and 29 to 34 and Enterovirus 68 to 71. Preferably, the Enterovirus is poliovirus. Enterovirus antigens are preferably selected from one or more of the following Capsid proteins VP1, VP2, VP3 and VP4. Commercially available polio vaccines include Inactivated Polio Vaccine (IPV) and Oral poliovirus vaccine (OPV).

[175] Heparnavirus: Viral antigens may be derived from an Heparnavirus, such as Hepatitis A virus (HAV). Commercially available HAV vaccines include inactivated HAV vaccine.

[176] Togavirus: Viral antigens may be derived from a Togavirus, such as a Rubivirus, an Alphavirus, or an Arterivirus. Antigens derived from Rubivirus, such as Rubella virus, are preferred. Togavirus antigens may be selected from E1, E2, E3, C, NSP-1, NSPO-2, NSP-3 or NSP-4. Togavirus antigens are preferably selected from E1, E2 or E3. Commercially available Rubella vaccines include a live cold-adapted virus, typically in combination with mumps and measles vaccines (MMR).

[177] Flavivirus: Viral antigens may be derived from a Flavivirus, such as Tick-borne encephalitis (TBE), Dengue (types 1, 2, 3 or 4), Yellow Fever, Japanese encephalitis, West Nile encephalitis, St. Louis encephalitis, Russian spring-summer encephalitis, Powassan encephalitis. Flavivirus antigens may be selected from PrM, M, C, E, NS-1, NS-2a, NS2b, NS3, NS4a, NS4b, and NS5. Flavivirus antigens are preferably selected from PrM, M and E. Commercially available TBE vaccine include inactivated virus vaccines.

[178] Pestivirus: Viral antigens may be derived from a Pestivirus, such as Bovine viral diarrhea (BVDV), Classical swine fever (CSFV) or Border disease (BDV).

[179] Hepadnavirus: Viral antigens may be derived from a Hepadnavirus, such as Hepatitis B virus. Hepadnavirus antigens may be selected from surface antigens (L, M and S), core

antigens (HBc, HBe). Commercially available HBV vaccines include subunit vaccines comprising the surface antigen S protein.

[180] Hepatitis C virus: Viral antigens may be derived from a Hepatitis C virus (HCV). HCV antigens may be selected from one or more of E1, E2, E1/E2, NS345 polyprotein, NS 345-core polyprotein, core, and/or peptides from the nonstructural regions (Houghton et al., Hepatology (1991) 14:381).

[181] Rhabdovirus: Viral antigens may be derived from a Rhabdovirus, such as a Lyssavirus (Rabies virus) and Vesiculovirus (VSV). Rhabdovirus antigens may be selected from glycoprotein (G), nucleoprotein (N), large protein (L), nonstructural proteins (NS). Commercially available Rabies virus vaccine comprise killed virus grown on human diploid cells or fetal rhesus lung cells.

[182] Caliciviridae; Viral antigens may be derived from Calciviridae, such as Norwalk virus, and Norwalk-like Viruses, such as Hawaii Virus and Snow Mountain Virus.

[183] Coronavirus: Viral antigens may be derived from a Coronavirus, SARS, Human respiratory coronavirus, Avian infectious bronchitis (IBV), Mouse hepatitis virus (MHV), and Porcine transmissible gastroenteritis virus (TGEV). Coronavirus antigens may be selected from spike (S), envelope (E), matrix (M), nucleocapsid (N), and Hemagglutinin-esterase glycoprotein (HE). Preferably, the Coronavirus antigen is derived from a SARS virus. SARS viral antigens are described in WO 04/92360;

[184] Retrovirus: Viral antigens may be derived from a Retrovirus, such as an Oncovirus, a Lentivirus or a Spumavirus. Oncovirus antigens may be derived from HTLV-1, HTLV-2 or HTLV-5. Lentivirus antigens may be derived from HIV-1 or HIV-2. Retrovirus antigens may be selected from gag, pol, env, tax, tat, rex, rev, nef, vif, vpu, and vpr. HIV antigens may be selected from gag (p24gag and p55gag), env (gp160 and gp41), pol, tat, nef, rev vpu, miniproteins, (preferably p55 gag and gp140v delete). HIV antigens may be

derived from one or more of the following strains: HIVIIIb, HIVSF2, HIVLAV, HIVLAI, HIVMN, HIV-1CM235, HIV-1US4.

[185] Reovirus: Viral antigens may be derived from a Reovirus, such as an Orthoreovirus, a Rotavirus, an Orbivirus, or a Coltivirus. Reovirus antigens may be selected from structural proteins λ1, λ2, λ3, μ1, μ2, σ1, σ2, or σ3, or nonstructural proteins σNS, μNS, or σ1s. Preferred Reovirus antigens may be derived from a Rotavirus. Rotavirus antigens may be selected from VP1, VP2, VP3, VP4 (or the cleaved product VP5 and VP8), NSP 1, VP6, NSP3, NSP2, VP7, NSP4, or NSP5. Preferred Rotavirus antigens include VP4 (or the cleaved product VP5 and VP8), and VP7.

[186] Parvovirus: Viral antigens may be derived from a Parvovirus, such as Parvovirus B19. Parvovirus antigens may be selected from VP-1, VP-2, VP-3, NS-1 and NS-2. Preferably, the Parvovirus antigen is capsid protein VP-2.

[187] Delta hepatitis virus (HDV): Viral antigens may be derived HDV, particularly δ-antigen from HDV (see, e.g., U.S. Patent No. 5,378,814).

[188] Hepatitis E virus (HEV): Viral antigens may be derived from HEV.

[189] Hepatitis G virus (HGV): Viral antigens may be derived from HGV.

[190] Human Herpesvirus: Viral antigens may be derived from a Human Herpesvirus, such as Herpes Simplex Viruses (HSV), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human Herpesvirus 6 (HHV6), Human Herpesvirus 7 (HHV7), and Human Herpesvirus 8 (HHV8). Human Herpesvirus antigens may be selected from immediate early proteins (α), early proteins (β), and late proteins (γ). HSV antigens may be derived from HSV-1 or HSV-2 strains. HSV antigens may be selected from glycoproteins gB, gC, gD and gH, fusion protein (gB), or immune escape proteins (gC, gE, or gI). VZV antigens may be selected from core, nucleocapsid, tegument, or envelope proteins. A live attenuated VZV vaccine is commercially available. EBV antigens may be selected from early antigen (EA) proteins, viral capsid antigen (VCA),

and glycoproteins of the membrane antigen (MA). CMV antigens may be selected from capsid proteins, envelope glycoproteins (such as gB and gH), and tegument proteins

[191] Papovaviruses: Antigens may be derived from Papovaviruses, such as Papillomaviruses and Polyomaviruses. Papillomaviruses include HPV serotypes 1, 2, 4, 5, 6, 8, 11, 13, 16, 18, 31, 33, 35, 39, 41, 42, 47, 51, 57, 58, 63 and 65. Preferably, HPV antigens are derived from serotypes 6, 11, 16 or 18. HPV antigens may be selected from capsid proteins (L1) and (L2), or E1 – E7, or fusions thereof. HPV antigens are preferably formulated into virus-like particles (VLPs). Polyomyavirus viruses include BK virus and JK virus. Polyomavirus antigens may be selected from VP1, VP2 or VP3.

[192] Further provided are antigens, compositions, methods, and microbes included in Vaccines, 4th Edition (Plotkin and Orenstein ed. 2004); Medical Microbiology 4th Edition (Murray et al. ed. 2002); Virology, 3rd Edition (W.K. Joklik ed. 1988); Fundamental Virology, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991), which are contemplated in conjunction with the compositions of the present invention.

C. Fungal Antigens

[193] Fungal antigens for use in the invention may be derived from one or more of the fungi set forth below.

[194] Fungal antigens may be derived from Dermatophytres, including: Epidermophyton floccusum, Microsporum audouini, Microsporum canis, Microsporum distortum, Microsporum equinum, Microsporum gypsum, Microsporum nanum, Trichophyton concentricum, Trichophyton equinum, Trichophyton gallinae, Trichophyton gypseum, Trichophyton megnini, Trichophyton mentagrophytes, Trichophyton quinckeanum, Trichophyton rubrum, Trichophyton schoenleini, Trichophyton tonsurans, Trichophyton verrucosum, T. verrucosum var. album, var. discoides, var. ochraceum, Trichophyton violaceum, and/or Trichophyton faviforme.

[195] Fungal pathogens may be derived from Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus sydowi, Aspergillus flavatus, Aspergillus glaucus, Blastoschizomyces capitatus, Candida albicans, Candida enolase, Candida tropicalis, Candida glabrata, Candida krusei, Candida parapsilosis, Candida stellatoidea, Candida kusei, Candida parakwsei, Candida lusitaniae, Candida pseudotropicalis, Candida guilliermondi, Cladosporium carrionii, Coccidioides immitis, Blastomyces dermatidis, Cryptococcus neoformans, Geotrichum clavatum, Histoplasma capsulatum, Klebsiella pneumoniae, Paracoccidioides brasiliensis, Pneumocystis carinii, Pythiumn insidiosum, Pityrosporum ovale, Sacharomyces cerevisae, Saccharomyces boulardii, Saccharomyces pombe, Scedosporium apiosperum, Sporothrix schenckii, Trichosporon beigelii, Toxoplasma gondii, Penicillium marneffei, Malassezia spp., Fonsecaea spp., Wangiella spp., Sporothrix spp., Basidiobolus spp., Conidiobolus spp., Rhizopus spp, Mucor spp, Absidia spp, Mortierella spp, Cunninghamella spp, Saksenaea spp., Alternaria spp, Curvularia spp, Helminthosporium spp, Fusarium spp, Aspergillus spp, Penicillium spp, Monolinia spp, Rhizoctonia spp, Paecilomyces spp, Pithomyces spp, and Cladosporium spp.

[196] Processes for producing a fungal antigens are well known in the art (see US Patent No. 6,333,164). In a preferred method a solubilized fraction extracted and separated from an insoluble fraction obtainable from fungal cells of which cell wall has been substantially removed or at least partially removed, characterized in that the process comprises the steps of: obtaining living fungal cells; obtaining fungal cells of which cell wall has been substantially removed or at least partially removed; bursting the fungal cells of which cell wall has been substantially removed or at least partially removed; obtaining an insoluble fraction; and extracting and separating a solubilized fraction from the insoluble fraction.

D. STD Antigens

[197] The compositions of the invention may include one or more antigens derived from a sexually transmitted disease (STD). Such antigens may provide for prophylactis or

therapy for STD's such as chlamydia, genital herpes, hepatits (such as HCV), genital warts, gonorrhoea, syphilis and/or chancroid (See, WO00/15255). Antigens may be derived from one or more viral or bacterial STD's. Viral STD antigens for use in the invention may be derived from, for example, HIV, herpes simplex virus (HSV-1 and HSV-2), human papillomavirus (HPV), and hepatitis (HCV). Bacterial STD antigens for use in the invention may be derived from, for example, Neiserria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Haemophilus ducreyi, E. coli, and Streptococcus agalactiae. Examples of specific antigens derived from these pathogens are described above.

E. Respiratory Antigens

[198] The compositions of the invention may include one or more antigens derived from a pathogen which causes respiratory disease. For example, respiratory antigens may be derived from a respiratory virus such as Orthomyxoviruses (influenza), Pneumovirus (RSV), Paramyxovirus (PIV), Morbillivirus (measles), Togavirus (Rubella), VZV, and Coronavirus (SARS). Respiratory antigens may be derived from a bacteria which causes respiratory disease, such as Streptococcus pneumoniae, Pseudomonas aeruginosa, Bordetella pertussis, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Chlamydia pneumoniae, Bacillus anthracis, and Moraxella catarrhalis. Examples of specific antigens derived from these pathogens are described above.

F. Pediatric Vaccine Antigens

[199] The compositions of the invention may include one or more antigens suitable for use in pediatric subjects. Pediatric subjects are typically less than about 3 years old, or less than about 2 years old, or less than about 1 years old. Pediatric antigens may be administered multiple times over the course of 6 months, 1, 2 or 3 years. Pediatric antigens may be derived from a virus which may target pediatric populations and/or a virus from which pediatric populations are susceptible to infection. Pediatric viral antigens include antigens derived from one or more of Orthomyxovirus (influenza), Pneumovirus (RSV),

Paramyxovirus (PIV and Mumps), Morbillivirus (measles), Togavirus (Rubella), Enterovirus (polio), HBV, Coronavirus (SARS), and Varicella-zoster virus (VZV), Epstein Barr virus (EBV). Pediatric bacterial antigens include antigens derived from one or more of Streptococcus pneumoniae, Neisseria meningitides, Streptococcus pyogenes (Group A Streptococcus), Moraxella catarrhalis, Bordetella pertussis, Staphylococcus aureus, Clostridium tetani (Tetanus), Cornynebacterium diphtheriae (Diphtheria), Haemophilus influenzae B (Hib), Pseudomonas aeruginosa, Streptococcus agalactiae (Group B Streptococcus), and E. coli. Examples of specific antigens derived from these pathogens are described above.

G. Antigens suitable for use in Elderly or Immunocompromised Individuals

[200] The compositions of the invention may include one or more antigens suitable for use in elderly or immunocompromised individuals. Such individuals may need to be vaccinated more frequently, with higher doses or with adjuvanted formulations to improve their immune response to the targeted antigens. Antigens which may be targeted for use in Elderly or Immunocompromised individuals include antigens derived from one or more of the following pathogens: Neisseria meningitides, Streptococcus pneumoniae, Streptococcus pyogenes (Group A Streptococcus), Moraxella catarrhalis, Bordetella pertussis, Staphylococcus aureus, Staphylococcus epidermis, Clostridium tetani (Tetanus), Cornynebacterium diphtheriae (Diphtheria), Haemophilus influenzae B (Hib), Pseudomonas aeruginosa, Legionella pneumophila, Streptococcus agalactiae (Group B Streptococcus), Enterococcus faecalis, Helicobacter pylori, Clamydia pneumoniae, Orthomyxovirus (influenza), Pneumovirus (RSV), Paramyxovirus (PIV and Mumps), Morbillivirus (measles), Togavirus (Rubella), Enterovirus (polio), HBV, Coronavirus (SARS), Varicella-zoster virus (VZV), Epstein Barr virus (EBV), Cytomegalovirus (CMV). Examples of specific antigens derived from these pathogens are described above.

H. Antigens suitable for use in Adolescent Vaccines

[201] The compositions of the invention may include one or more antigens suitable for use in adolescent subjects. Adolescents may be in need of a boost of a previously administered pediatric antigen. Pediatric antigens which may be suitable for use in adolescents are described above. In addition, adolescents may be targeted to receive antigens derived from an STD pathogen in order to ensure protective or therapeutic immunity before the beginning of sexual activity. STD antigens which may be suitable for use in adolescents are described above.

I. Antigen Formulations

[202] In other aspects of the invention, methods of producing microparticles having adsorbed antigens are provided. The methods comprise: (a) providing an emulsion by dispersing a mixture comprising (i) water, (ii) a detergent, (iii) an organic solvent, and (iv) a biodegradable polymer selected from the group consisting of a poly(α-hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride, and a polycyanoacrylate. The polymer is typically present in the mixture at a concentration of about 1% to about 30% relative to the organic solvent, while the detergent is typically present in the mixture at a weight-to-weight detergent-to-polymer ratio of from about 0.00001:1 to about 0.1:1 (more typically about 0.0001:1 to about 0.1:1, about 0.001:1 to about 0.1:1, or about 0.005:1 to about 0.1:1); (b) removing the organic solvent from the emulsion; and (c) adsorbing an antigen on the surface of the microparticles. In certain embodiments, the biodegradable polymer is present at a concentration of about 3% to about 10% relative to the organic solvent.

[203] Microparticles for use herein will be formed from materials that are sterilizable, non-toxic and biodegradable. Such materials include, without limitation, poly(α-hydroxy acid), polyhydroxybutyric acid, polycaprolactone, polyorthoester, polyanhydride, PACA, and polycyanoacrylate. Preferably, microparticles for use with the present invention are derived from a poly(α-hydroxy acid), in particular, from a poly(lactide) ("PLA") or a

copolymer of D,L-lactide and glycolide or glycolic acid, such as a poly(D,L-lactide-co-glycolide) ("PLG" or "PLGA"), or a copolymer of D,L-lactide and caprolactone. The microparticles may be derived from any of various polymeric starting materials which have a variety of molecular weights and, in the case of the copolymers such as PLG, a variety of lactide:glycolide ratios, the selection of which will be largely a matter of choice, depending in part on the coadministered macromolecule. These parameters are discussed more fully below.

[204] Further antigens may also include an outer membrane vesicle (OMV) preparation.

[205] Additional formulation methods and antigens (especially tumor antigens) are provided in U.S. Patent Serial No. 09/581,772.

J. Antigen References

[206] The following references include antigens useful in conjunction with the compositions of the present invention:

1 International patent application WO99/24578
2 International patent application WO99/36544.
3 International patent application WO99/57280.
4 International patent application WO00/22430.
5 Tettelin et al. (2000) Science 287:1809-1815.
6 International patent application WO96/29412.
7 Pizza et al. (2000) Science 287:1816-1820.
8 PCT WO 01/52885.
9 Bjune et al. (1991) Lancet 338(8775).
10 Fuskasawa et al. (1999) Vaccine 17:2951-2958.
11 Rosenqist et al. (1998) Dev. Biol. Strand 92:323-333.
12 Constantino et al. (1992) Vaccine 10:691-698.
13 Constantino et al. (1999) Vaccine 17:1251-1263.
14 Watson (2000) Pediatr Infect Dis J 19:331-332.
15 Rubin (20000) Pediatr Clin North Am 47:269-285,v.
16 Jedrzejas (2001) Microbiol Mol Biol Rev 65:187-207.
17 International patent application filed on 3rd July 2001 claiming priority from GB-0016363.4;WO 02/02606; PCT IB/01/00166.
18 Kalman et al. (1999) Nature Genetics 21:385-389.
19 Read et al. (2000) Nucleic Acids Res 28:1397-406.

20 Shirai et al. (2000) J. Infect. Dis 181(Suppl 3):S524-S527.
21 International patent application WO99/27105.
22 International patent application WO00/27994.
23 International patent application WO00/37494.
24 International patent application WO99/28475.
25 Bell (2000) Pediatr Infect Dis J 19:1187-1188.
26 Iwarson (1995) APMIS 103:321-326.
27 Gerlich et al. (1990) Vaccine 8 Suppl:S63-68 & 79-80.
28 Hsu et al. (1999) Clin Liver Dis 3:901-915.
29 Gastofsson et al. (1996) N. Engl. J. Med. 334-:349-355.
30 Rappuoli et al. (1991) TIBTECH 9:232-238.
31 Vaccines (1988) eds. Plotkin & Mortimer. ISBN 0-7216-1946-0.
32 Del Guidice et al. (1998) Molecular Aspects of Medicine 19:1-70.
33 International patent application WO93/018150.
34 International patent application WO99/53310.
35 International patent application WO98/04702.
36 Ross et al. (2001) Vaccine 19:135-142.
37 Sutter et al. (2000) Pediatr Clin North Am 47:287-308.
38 Zimmerman & Spann (1999) Am Fan Physician 59:113-118, 125-126.
39 Dreensen (1997) Vaccine 15 Suppl"S2-6.
40 MMWR Morb Mortal Wkly rep 1998 Jan 16:47(1):12, 9.
41 McMichael (2000) Vaccine19 Suppl 1:S101-107.
42 Schuchat (1999) Lancer 353(9146):51-6.
43 GB patent applications 0026333.5, 0028727.6 & 0105640.7.
44 Dale (1999) Infect Disclin North Am 13:227-43, viii.
45 Ferretti et al. (2001) PNAS USA 98: 4658-4663.
46 Kuroda et al. (2001) Lancet 357(9264):1225-1240; see also pages 1218-1219.
47 Ramsay et al. (2001) Lancet 357(9251):195-196.
48 Lindberg (1999) Vaccine 17 Suppl 2:S28-36.
49 Buttery & Moxon (2000) J R Coil Physicians Long 34:163-168.
50 Ahmad & Chapnick (1999) Infect Dis Clin North Am 13:113-133, vii.
51 Goldblatt (1998) J. Med. Microbiol. 47:663-567.
52 European patent 0 477 508.
53 U.S. Patent No. 5,306,492.
54 International patent application WO98/42721.
55 Conjugate Vaccines (eds. Cruse et al.) ISBN 3805549326, particularly vol. 10:48-114.
56 Hermanson (1996) Bioconjugate Techniques ISBN: 012323368 & 012342335X.
57 European patent application 0372501.
58 European patent application 0378881.
59 European patent application 0427347.
60 International patent application WO93/17712.
61 International patent application WO98/58668.
62 European patent application 0471177.

63 International patent application WO00/56360.
64 International patent application WO00/67161.

[207] The contents of all of the above cited patents, patent applications and journal articles are incorporated by reference as if set forth fully herein.

[208] Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity. See Ramsay *et al.* (2001) Lancet 357(9251):195-196; Lindberg (1999) Vaccine 17 Suppl 2:S28-36; Buttery & Moxon (2000) J R Coll Physicians Lond 34:163-168; Ahmad & Chapnick (1999) Infect Dis Clin North Am 13:113-133, vii; Goldblatt (1998) J. Med. Microbiol. 47:563-567; European patent 0 477 508; US Patent No. 5,306,492; WO98/42721; Conjugate Vaccines (eds. Cruse *et al.*) ISBN 3805549326, particularly vol. 10:48-114; Hermanson (1996) Bioconjugate Techniques ISBN: 0123423368 or 012342335X. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM197 diphtheria toxoid is particularly preferred.

[209] Other carrier polypeptides include the *N. meningitidis* outer membrane protein (EP-A-0372501), synthetic peptides (EP-A-0378881 and EP-A 0427347), heat shock proteins (WO 93/17712 and WO 94/03208), pertussis proteins (WO 98/58668 and EP A 0471177), protein D from H. influenzae (WO 00/56360), cytokines (WO 91/01146), lymphokines, hormones, growth factors, toxin A or B from C. difficile (WO 00/61761), iron-uptake proteins (WO 01/72337), etc. Where a mixture comprises capsular saccharide from both serigraphs A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.*, 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

[210] Toxic protein antigens may be detoxified where necessary *e.g.*, detoxification of pertussis toxin by chemical and/or genetic means.

Pharmaceutically acceptable carriers

[211] Compositions of the invention will typically, in addition to the components mentioned above, comprise one or more "pharmaceutically acceptable carriers." These include any carrier which does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers typically are large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. A composition may also contain a diluent, such as water, saline, glycerol, etc. Additionally, an auxiliary substance, such as a wetting or emulsifying agent, pH buffering substance, and the like, may be present. A thorough discussion of pharmaceutically acceptable components is available in Gennaro (2000) Remington: The Science and Practice of Pharmacy. 20th ed., ISBN: 0683306472.

Immunoregulatory Agents

Adjuvants

[212] Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Adjuvants for use with the invention include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

[213] Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminum salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulfates, etc. (*e.g.* see chapters 8 & 9 of Vaccine Design... (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.), or mixtures of different mineral compounds (*e.g.* a mixture of a phosphate and a hydroxide adjuvant, optionally with an

excess of the phosphate), with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, etc.), and with adsorption to the salt(s) being preferred. The mineral containing compositions may also be formulated as a particle of metal salt (WO00/23105).

[214] Aluminum salts may be included in vaccines of the invention such that the dose of Al^{3+} is between 0.2 and 1.0 mg per dose.

[215] In one embodiment the aluminum based adjuvant for use in the present invention is alum (aluminum potassium sulfate ($AlK(SO_4)_2$)), or an alum derivative, such as that formed in-situ by mixing an antigen in phosphate buffer with alum, followed by titration and precipitation with a base such as ammonium hydroxide or sodium hydroxide.

[216] Another aluminum-based adjuvant for use in vaccine formulations of the present invention is aluminum hydroxide adjuvant ($Al(OH)_3$) or crystalline aluminum oxyhydroxide (AlOOH), which is an excellent adsorbant, having a surface area of approximately $500m^2/g$. Alternatively, aluminum phosphate adjuvant ($AlPO_4$) or aluminum hydroxyphosphate, which contains phosphate groups in place of some or all of the hydroxyl groups of aluminum hydroxide adjuvant is provided. Preferred aluminum phosphate adjuvants provided herein are amorphous and soluble in acidic, basic and neutral media.

[217] In another embodiment the adjuvant of the invention comprises both aluminum phosphate and aluminum hydroxide. In a more particular embodiment thereof, the adjuvant has a greater amount of aluminum phosphate than aluminum hydroxide, such as a ratio of 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or greater than 9:1, by weight aluminum phosphate to aluminum hydroxide. More particular still, aluminum salts in the vaccine are present at 0.4 to 1.0 mg per vaccine dose, or 0.4 to 0.8 mg per vaccine dose, or 0.5 to 0.7 mg per vaccine dose, or about 0.6 mg per vaccine dose.

[218] Generally, the preferred aluminum-based adjuvant(s), or ratio of multiple aluminum-based adjuvants, such as aluminum phosphate to aluminum hydroxide is selected by optimization of electrostatic attraction between molecules such that the antigen carries an opposite charge as the adjuvant at the desired pH. For example, aluminum phosphate adjuvant (isoelectric point = 4) adsorbs lysozyme, but not albumin at pH 7.4. Should albumin be the target, aluminum hydroxide adjuvant would be selected (iep 11.4). Alternatively, pretreatment of aluminum hydroxide with phosphate lowers its isoelectric point, making it a preferred adjuvant for more basic antigens.

B. Oil-Emulsions

[219] Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% TWEEN™ 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Podda, Vaccine (2001) 19: 2673-2680; Frey *et al.*, Vaccine (2003) 21:4234-4237. MF59 is used as the adjuvant in the FLUAD™ influenza virus trivalent subunit vaccine.

[220] Particularly preferred adjuvants for use in the compositions are submicron oil-in-water emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v TWEEN™ 80□ (polyoxyelthylenesorbitan monooleate), and/or 0.25-1.0% SPAN 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-huydroxyphosphophoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "MF59" (International Publication No. WO90/14837; US Patent Nos. 6,299,884 and 6,451,325, and Ott *et al.*, in Vaccine Design: The Subunit and Adjuvant Approach (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (*e.g.* 4.3%), 0.25-0.5% w/v TWEEN™ 80, and 0.5% w/v SPAN 85™ and optionally

contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 μg/dose, more preferably 0-250 μg/dose and most preferably, 0-100 μg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 μg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v TWEEN™ 80, and 0.75% w/v SPAN 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% TWEEN™ 80, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 μg MTP-PE per dose.

[221] Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in WO90/14837 and U.S. Patents 6,299,884 and 6,45 1,325.

[222] Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

[223] Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponins isolated from the bark of the Quillaia saponaria Molina tree have been widely studied as adjuvants. Saponins can also be commercially obtained from Smilax ornata (sarsaprilla), Gypsophilla paniculata (brides veil), and Saponaria officianalis (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

[224] Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-TLC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO96/33739).

[225] Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexes (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP0109942, WO96/11711 and WO96/33739. Optionally, the ISCOMS may be devoid of (an) additional detergent(s). See WO00/07621.

[226] A review of the development of saponin based adjuvants can be found in Barr, *et al.*, Advanced Drug Delivery Reviews (1998) 32:247-271. *See also* Sjolander, *et al.*, Advanced Drug Delivery Reviews (1998) 32:321-338.

D. Virosomes and Virus Like Particles (VLPs)

[227] Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat

proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO03/024480, WO03/024481, and Niikura *et al.*, Virology (2002) 293:273-280; Lenz *et al.*, Journal of Immunology (2001) 5246-5355; Pinto, *et al.*, Journal of Infectious Diseases (2003) 188:327-338; and Gerber *et al.*, Journal of Virology (2001) 75(10):4752-4760. Virosomes are discussed further in, for example, Gluck *et al.*, Vaccine (2002) 20:B10 –B16. Immunopotentiating reconstituted influenza virosomes (IRIV) are used as the subunit antigen delivery system in the intranasal trivalent INFLEXAL™ product {Mischler & Metcalfe (2002) Vaccine 20 Suppl 5:B17-23} and the INFLUVAC PLUS™ product.

E. Bacterial or Microbial Derivatives

[228] Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)

[229] Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC 529. See Johnson *et al.* (1999) Bioorg Med Chem Lett 9:2273-2278.

(2) Lipid A Derivatives

[230] Lipid A derivatives include derivatives of lipid A from Escherichia coli such as OM-174. OM-174 is described for example in Meraldi *et al.*, Vaccine (2003) 21:2485-2491; and Pajak, *et al.*, Vaccine (2003) 21:836-842.

(3) Immunostimulatory oligonucleotides

[231] Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

[232] The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See Kandimalla, *et al.*, Nucleic Acids Research (2003) 31(9): 2393-2400; WO02/26757 and WO99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Krieg, Nature Medicine (2003) 9(7): 831-835; McCluskie, *et al.*, FEMS Immunology and Medical Microbiology (2002) 32:179-185; WO98/40100; US Patent No. 6,207,646; US Patent No. 6,239,116 and US Patent No. 6,429,199.

[233] The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See Kandimalla, *et al.*, Biochemical Society Transactions (2003) 31 (part 3): 654-658. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in Blackwell, *et al.*, J. Immunol. (2003) 170(8):4061-4068; Krieg, TRENDS in Immunology (2002) 23(2): 64-65 and WO01/95935. Preferably, the CpG is a CpG-A ODN.

[234] Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, Kandimalla, *et al.*, BBRC (2003) 306:948-953; Kandimalla, *et al.*, Biochemical Society Transactions (2003) 31(part 3):664-658; Bhagat *et al.*, BBRC (2003) 300:853-861 and WO03/035836.

(4) ADP-ribosylating toxins and detoxified derivatives thereof.

[235] Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (*i.e.*, *E. coli* heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO95/17211 and as parenteral adjuvants in WO98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivatives thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in the following references: Beignon, *et al.*, Infection and Immunity (2002) 70(6):3012-3019; Pizza, *et al.*, Vaccine (2001) 19:2534-2541; Pizza, *et al.*, Int. J. Med. Microbiol (2000) 290(4-5):455-461; Scharton-Kersten *et al.*, Infection and Immunity (2000) 68(9):5306-5313; Ryan *et al.*, Infection and Immunity (1999) 67(12):6270-6280; Partidos *et al.*, Immunol. Lett. (1999) 67(3):209-216; Peppoloni *et al.*, Vaccines (2003) 2(2):285-293; and Pine *et al.*, (2002) J. Control Release (2002) 85(1-3):263-270. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini *et al.*, Mol. Microbiol (1995) 15(6):1165-1167.

F. Bioadhesives and Mucoadhesives

[236] Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Singh *et al.* (2001) J. Cont. Rele. 70:267-276) or mucoadhesives such as cross-linked derivatives of polyacrylic acid, polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. *See* WO99/27960.

G. Microparticles

[237] Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, etc.), with poly(lactide co glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

[238] Examples of liposome formulations suitable for use as adjuvants are described in US Patent No. 6,090,406, US Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

[239] Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. WO99/52549. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (WO01/21207) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152).

[240] Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

[241] PCPP formulations are described, for example, in Andrianov *et al.*, "Preparation of hydrogel microspheres by coacervation of aqueous polyphophazene solutions",

Biomaterials (1998) 19(1-3):109-115 and Payne *et al.*, "Protein Release from Polyphosphazene Matrices", Adv. Drug. Delivery Review (1998) 31(3):185-196.

K. Muramyl peptides

[242] Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-l-alanyl-d-isoglutamine (nor-MDP), and N acetylmuramyl-l-alanyl-d-isoglutaminyl-l-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinoline Compounds.

[243] Examples of imidazoquinoline compounds suitable for use adjuvants in the invention include Imiquimod and its analogues, described further in Stanley, Clin Exp Dermatol (2002) 27(7):571-577; Jones, Curr Opin Investig Drugs (2003) 4(2):214-218; and U.S. Patents 4,689,338, 5,389,640, 5,268,376, 4,929,624, 5,266,575, 5,352,784, 5,494,916, 5,482,936, 5,346,905, 5,395,937, 5,238,944, and 5,525,612.

M. Thiosemicarbazone Compounds.

[244] Examples of thiosemicarbazone compounds, as well as methods of formulating, manufacturing, and screening for compounds all suitable for use as adjuvants in the invention include those described in WO04/60308. The thiosemicarbazones are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF- α.

N. Tryptanthrin Compounds.

[245] Examples of tryptanthrin compounds, as well as methods of formulating, manufacturing, and screening for compounds all suitable for use as adjuvants in the invention include those described in WO04/64759. The tryptanthrin compounds are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF- α.

[246] The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (WO99/11241);

(2) a saponin (*e.g.*, QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) (see WO94/00153);

(3) a saponin (*e.g.*, QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol;

(4) a saponin (*e.g.*, QS21) + 3dMPL + IL 12 (optionally + a sterol) (WO98/57659);

(5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (See European patent applications 0835318, 0735898 and 0761231);

(6) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(7) RibiTM adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

(9) one or more mineral salts (such as an aluminum salt) + an immunostimulatory oligonucleotide (such as a nucleotide sequence including a CpG motif).

O. Human Immunomodulators

[247] Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

[248] Aluminum salts and MF59 are preferred adjuvants for use with injectable influenza vaccines. Bacterial toxins and bioadhesives are preferred adjuvants for use with mucosally-delivered vaccines, such as nasal vaccines.

[249] The contents of all of the above cited patents, patent applications and journal articles are incorporated by reference as if set forth fully herein.

Therapeutic methods

[250] The invention provides methods for inducing or increasing an immune response to a GAS antigen using the compositions described above. The immune response is preferably protective and can include antibodies and/or cell-mediated immunity (including systemic and mucosal immunity). Immune responses include booster responses. Compositions comprising antibodies can be used to treat *S. pyogenes* infections.

[251] Teenagers and children, including toddles and infants, can receive a vaccine for prophylactic use; therapeutic vaccines typically are administered to teenagers or adults. A vaccine intended for children may also be administered to adults *e.g.*, to assess safety, dosage, immunogenicity, etc.

[252] Diseases caused by *Streptococcus pyogenes* which can be prevented or treated according to the invention include, but are not limited to, pharyngitis (such as streptococcal sore

throat), scarlet fever, impetigo, erysipelas, cellulitis, septicemia, toxic shock syndrome, necrotizing fasciitis, and sequelae such as rheumatic fever and acute glomerulonephritis. The compositions may also be effective against other streptococcal bacteria, *e.g.*, GBS.

Tests to determine the efficacy of the immune response

[253] One way of assessing efficacy of therapeutic treatment involves monitoring GAS infection after administration of the composition of the invention. One way of assessing efficacy of prophylactic treatment involves monitoring immune responses against the GAS antigens in the compositions of the invention after administration of the composition.

[254] Another way of assessing the immunogenicity of the component proteins of the immunogenic compositions of the present invention is to express the proteins recombinantly and to screen patient sera or mucosal secretions by immunoblot. A positive reaction between the protein and the patient serum indicates that the patient has previously mounted an immune response to the protein in question; *i.e.*, the protein is an immunogen. This method may also be used to identify immunodominant proteins and/or epitopes.

[255] Another way of checking efficacy of therapeutic treatment involves monitoring GAS infection after administration of the compositions of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses both systemically (such as monitoring the level of IgG1 and IgG2a production) and mucosally (such as monitoring the level of IgA production) against the GAS antigens in the compositions of the invention after administration of the composition. Typically, GAS serum specific antibody responses are determined post-immunization but pre-challenge whereas mucosal GAS-specific antibody body responses are determined post-immunization and post-challenge.

[256] The vaccine compositions of the present invention can be evaluated in in vitro and in vivo animal models prior to host, *e.g.*, human, administration. Particularly useful mouse models include those in which intraperitoneal immunization is followed by either intraperitoneal challenge or intranasal challenge. A model in which intraperitoneal immunization is followed by intraperitoneal challenge is illustrated in FIG. 13.

[257] The efficacy of immunogenic compositions of the invention can also be determined in vivo by challenging animal models of GAS infection, *e.g.*, guinea pigs or mice, with the immunogenic compositions. The immunogenic compositions may or may not be derived from the same serotypes as the challenge serotypes. Preferably the immunnogenic compositions are derivable from the same serotypes as the challenge serotypes. More preferably, the immunogenic composition and/or the challenge serotype are derivable from the group of GAS serotypes consisting of M1, M3, M23 and/or combinations thereof.

[258] In vivo efficacy models include but are not limited to: (i) a murine infection model using human GAS serotypes; (ii) a murine disease model which is a murine model using a mouse-adapted GAS strain, such as the M23 strain which is particularly virulent in mice, and (iii) a primate model using human GAS isolates.

[259] The immune response may be one or both of a TH1 immune response and a TH2 response. The immune response may be an improved or an enhanced or an altered immune response. The immune response may be one or both of a systemic and a mucosal immune response. Preferably the immune response is an enhanced system and/or mucosal response.

[260] An enhanced systemic and/or mucosal immunity is reflected in an enhanced TH1 and/or TH2 immune response. Preferably, the enhanced immune response includes an increase in the production of IgG1 and/or IgG2a and/or IgA.

[261] Preferably the mucosal immune response is a TH2 immune response. Preferably, the mucosal immune response includes an increase in the production of IgA.

[262] Activated TH2 cells enhance antibody production and are therefore of value in responding to extracellular infections. Activated TH2 cells may secrete one or more of IL-4, IL-5, IL-6, and IL-10. A TH2 immune response may result in the production of IgG1, IgE, IgA and memory B cells for future protection.

[263] A TH2 immune response may include one or more of an increase in one or more of the cytokines associated with a TH2 immune response (such as IL-4, IL-5, IL-6 and IL-10), or an increase in the production of IgG1, IgE, IgA and memory B cells. Preferably, the enhanced TH2 immune resonse will include an increase in IgG1 production.

[264] A TH1 immune response may include one or more of an increase in CTLs, an increase in one or more of the cytokines associated with a TH1 immune response (such as IL-2, IFNγ, and TNFβ), an increase in activated macrophages, an increase in NK activity, or an increase in the production of IgG2a. Preferably, the enhanced TH1 immune response will include an increase in IgG2a production.

[265] Immunogenic compositions of the invention, in particular, immunogenic composition comprising one or more GAS antigens of the present invention may be used either alone or in combination with other GAS antigens optionally with an immunoregulatory agent capable of eliciting a Th1 and/or Th2 response.

[266] The invention also comprises an immunogenic composition comprising one or more immunoregulatory agent, such as a mineral salt, such as an aluminium salt and an oligonucleotide containing a CpG motif. Most preferably, the immunogenic composition includes both an aluminium salt and an oligonucleotide containing a CpG motif. Alternatively, the immunogenic composition includes an ADP ribosylating toxin, such as a detoxified ADP ribosylating toxin and an oligonucleotide containing a CpG motif. Preferably, one or more of the immunoregulatory agents include an adjuvant. The

adjuvant may be selected from one or more of the group consisting of a TH1 adjuvant and TH2 adjuvant, further discussed below.

[267] The compositions of the invention will preferably elicit both a cell mediated immune response as well as a humoral immune response in order to effectively address a GAS infection. This immune response will preferably induce long lasting (*e.g.*, neutralizing) antibodies and a cell mediated immunity that can quickly respond upon exposure to one or more GAS antigens.

[268] In one particularly preferred embodiment, the immunogenic composition comprises one or more GAS antigen(s) which elicits a neutralizing antibody response and one or more GAS antigen(s) which elicit a cell mediated immune response. In this way, the neutralizing antibody response prevents or inhibits an initial GAS infection while the cell-mediated immune response capable of eliciting an enhanced Th1 cellular response prevents further spreading of the GAS infection. Preferably, the immunogenic composition comprises one or more GAS surface antigens and one or more GAS cytoplasmic antigens. Preferably the immunogenic composition comprises one or more GAS40 surface antigens or the like and one or other antigens, such as a cytoplasmic antigen capable of eliciting a Th1 cellular response.

[269] Compositions of the invention will generally be administered directly to a patient. The compositions of the present invention may be administered, either alone or as part of a composition, via a variety of different routes. Certain routes may be favored for certain compositions, as resulting in the generation of a more effective immune response, preferably a CMI response, or as being less likely to induce side effects, or as being easier for administration.

[270] Delivery methods include parenteral injection (*e.g.*, subcutaneous, intraperitoneal, intravenous, intramuscular, or interstitial injection) and rectal, oral (*e.g.*, tablet, spray), vaginal, topical, transdermal (*e.g.*, see WO 99/27961), transcutaneous (*e.g.*, see

WO02/074244 and WO02/064162), intranasal (*e.g.*, see WO03/028760), ocular, aural, and pulmonary or other mucosal administration.

[271] By way of example, the compositions of the present invention may be administered via a systemic route or a mucosal route or a transdermal route or it may be administered directly into a specific tissue. As used herein, the term "systemic administration" includes but is not limited to any parenteral routes of administration. In particular, parenteral administration includes but is not limited to subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, or intrasternal injection, intravenous, intraarterial, or kidney dialytic infusion techniques. Preferably, the systemic, parenteral administration is intramuscular injection. As used herein, the term "mucosal administration" includes but is not limited to oral, intranasal, intravaginal, intrarectal, intratracheal, intestinal and ophthalmic administration.

[272] Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunization schedule and/or in a booster immunization schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.*, a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc.

[273] The compositions of the invention may be prepared in various forms. For example, a composition can be prepared as an injectable, either as a liquid solution or a suspension. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.*, a lyophilized composition). A composition can be prepared for oral administration, such as a tablet or capsule, as a spray, or as a syrup (optionally flavored). A composition can be prepared for pulmonary administration, *e.g.*, as an inhaler, using a fine powder or a spray. A composition can be prepared as a suppository or pessary. A composition can be prepared for nasal, aural or ocular administration *e.g.*, as drops. A composition can be in kit form, designed such that a combined composition is

reconstituted just prior to administration to a patient. Such kits may comprise one or more GAS or other antigens in liquid form and one or more lyophilized antigens.

[274] Immunogenic compositions used as vaccines comprise an immunologically effective amount of GAS or other antigens (or nucleic acid molecules encoding the antigens) or antibodies, as well as any other components, as needed, such as antibiotics. An "immunologically effective amount" is an amount which, when administered to an individual, either in a single dose or as part of a series, increases a measurable immune response or prevents or reduces a clinical symptom.

[275] The immunogenic compositions of the present invention may be administered in combination with an antibiotic treatment regime. In one embodiment, the antibiotic is administered prior to administration of the antigen of the invention or the composition comprising the one or more GAS antigens of the invention.

[276] In another embodiment, the antibiotic is administered subsequent to the administration of the one or more surface-exposed and/or surface-associated GAS antigens of the invention or the composition comprising the one or more surface-exposed and/or surface-associated GAS antigens of the invention. Examples of antibiotics suitable for use in the treatment of a GAS infection include but are not limited to penicillin or a derivative thereof or clindamycin, cephalosporins, glycopeptides (*e.g.*, vancomycin), and cycloserine.

[277] The amount of active agent in a composition varies, however, depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.*, non-human primate, primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. The amount will fall in a relatively broad range which can be determined through routine trials.

Kits

[278] The invention also provides kits comprising one or more containers of compositions of the invention. Compositions can be in liquid form or can be lyophilized, as can individual antigens. Suitable containers for the compositions include, for example, bottles, vials, syringes, and test tubes. Containers can be formed from a variety of materials, including glass or plastic. A container may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle).

[279] The kit can further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, or dextrose solution. It can also contain other materials useful to the end-user, including other buffers, diluents, filters, needles, and syringes. The kit can also comprise a second or third container with another active agent, for example an antibiotic.

[280] The kit can also comprise a package insert containing written instructions for methods of inducing immunity against *S. pyogenes* or for treating *S. pyogenes* infections. The package insert can be an unapproved draft package insert or can be a package insert approved by the Food and Drug Administration (FDA) or other regulatory body.

[281] All patents, patent applications, and references cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Identification of surface-exposed GAS antigens

[282] A set of 73 proteins were identified in silico as surface-expressed proteins of the GASSF370 strain (M1) genome using BLAST, FASTa, MOTIFS, FINDPATTERNS,

PSORT, and searches of the ProDom, Pfam, and Blocks databases. These programs were used to predict features typical of surface-associated proteins, such as transmembrane domains, leader peptides, homologies to known surface proteins, lipoprotein signatures, outer membrane anchoring motifs, and host-cell binding domains such as RGD. The results are shown in Table 3.

[283] Commercially available *E. coli* expression vectors pET and pGex were used to express GAS antigens either as HIS-tagged proteins or as HIS-GST fusions, *i.e.*, an amino-terminal histidine tag and a carboxy terminal GST (wherever "GST" is not specified, only the HIS-tagged antigen was expressed. See Figures 4A and 4B. In some cases, urea was used to solubilize the antigen. Briefly, PCR reactions were performed to amplify GAS antigen coding sequences, then the amplified products were digested overnight. The digested PCR products were then purified and ligated overnight with either pET or pGEX vectors. *E. coli* strains BL21(DE3) and BL21 were transformed with the pGEX and pET ligation products, respectively, plated, and incubated at 37 °C. Two PCR-positive colonies from each transformation were inoculated and grown overnight. Protein expression in these clones was induced with IPTG and assessed by SDS-PAGE analysis of *E. coli* extracts. Glycerol batches of the GAS antigen-expressing clones were then prepared.

[284] Two of the identified proteins (GAS87, 171) were not expressed. Mice were immunized with 70 of the other proteins. Sera of these mice were used for FACS analysis on native bacterial cells, and surface exposure was tested on 20 GAS strains of different M types (see Table 2). The presence or absence of each protein on the bacterial cell surface was assessed by calculating the difference (Delta Mean) between the FACS value obtained with the immune serum and that obtained with the preimmune serum. An arbitrary cut-off of Delta Mean $\geq$ 80 was used to classify a protein as "surface exposed." Three of the tested antigens did not meet this threshold (GAS88, 208, and 210). The results for the other GAS antigens tested are shown in Tables 4A-4R and FIG. 2.

EXAMPLE 2

GAS40 protein identity in different GAS strains

[285] Genomic DNAs were prepared from GAS strains of different M types, and the complete protein sequence of the full-length GAS40 antigen was obtained. The results are shown in FIG. 1 and in Table 5.

EXAMPLE 3

Demonstration that GAS40 proteins are surface-exposed

[286] FACS analysis was carried out as described in Example 1 to demonstrate that GAS40 proteins are surface-exposed. The results are shown in FIG. 2.

EXAMPLE 4

FACS analysis of various GAS40 antigens

[287] Mice were immunized with various GAS40 antigens. Sera of these mice were used for FACS analysis on native bacterial cells of various *S. pyogenes* strains. The ability of these antisera to detect GAS40 protein on the bacterial cell surface was assessed by calculating the difference between the FACS value obtained with the immune sera and that obtained with the preimmune sera. The results are shown in FIGS. 3-11.

[288] "40 native" (FIG. 3) is the GAS40 protein having the amino acid sequence shown in SEQ ID NO:17 and is encoded by a nucleotide sequence derived from the genomic sequence of the SF 370 strain.

[289] "GST40" (FIGS. 4A-4B) is a hybrid GAS40 antigen with glutathione-S-transferase in place of the leader sequence at its N terminus. The N-terminal amino acids LVPRGSHM (SEQ ID NO:963) and the C-terminal amino acids AAALEHHHHHH (SEQ ID NO:964) belong to the GST vector pGEXNNH.

[290] "40a" (FIGS. 5A-5B) is a GAS40 antigen with a HIS tag but without the leader and hydrophobic sequences (SEQ ID NO:235). The nucleotide sequence shown in SEQ ID NO:892 was cloned into vector pET21b+ (Novagen) using the NdeI and NotI restriction sites. The carboxyl terminal 12 amino acids were introduced with the vector. Codon 824 (AGA in the wild-type sequence) was mutagenized to CGT.

[291] "40aCH" (FIGS. 6A-6B) is a GAS40 antigen with a HIS tag at its carboxyl terminus and two additional amino acids at its N terminus. Three nucleotide changes introduced with the cloning of its coding sequence into the pSM214gCH shuttle vector (at nucleotides 198, 222, and 1115). One amino acid (amino acid 372) was changed from Phe to Ser.

[292] "40/117" (FIG. 7) is a GAS40 hybrid antigen in which the GAS40 protein is placed to the N-terminus of the GAS117 protein and a HIS tag is added to the C terminus of the GAS117 protein (SEQ ID NO:234). SEQ ID NO:891 is a nucleotide sequence which codes for this antigen.

[293] "117/40" (FIG. 8) is a GAS40 hybrid antigen in which GAS117 to GAS40 by the linker sequence YASGGGS (SEQ ID NO:278). Its amino acid sequence is shown in SEQ ID NO:233; a coding sequence is shown in SEQ ID NO:890.

[294] "40aRR" (FIGS. 9A-9B) is similar to "40a" except that two additional AGA codons (334 and 335) in the coding sequence were mutated to CGT.

[295] "40aNH" (FIGS. 10A-10B) is a GAS40 antigen with the HIS tag at its N terminus. Its coding sequence was cloned into the *E. coli*/B. subtilis expression shuttle vector pSM214gNH, which uses a constitutive promoter instead of an IPTG inducible promoter. The amino terminal nine amino acids are introduced with the cloning. It contains two nucleotide changes which most likely occurred during PCR amplification and do not result in amino acid changes (nucleotides 356 and 1547).

[296] "40aRRNH" (FIGS. 11A-11B) is similar to "40aNH" except that codons 1034 and 1035 were modified to CGT.

EXAMPLE 5

Immunization of mice

[297] Groups of 10 CD1 female mice aged between 6 and 7 weeks are immunized with two or more GAS antigens of the invention, (20 μg of each recombinant GAS antigen), suspended in 100 μl of suitable solution. Each group received 3 doses at days 0, 21, and 45. Immunization was performed through intra-peritoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and Incomplete Freund's Adjuvant (IFA) for the following two doses. In each immunization scheme negative and positive control groups were used. See FIG. 13.

[298] For the negative control group, mice were immunized with *E. coli* proteins eluted from the purification columns following processing of total bacterial extract from an *E. coli* strain containing either the pET21b or the pGEX-NNH vector (thus expressing GST only) without any cloned GAS ORF (groups can be indicated as HisStop or GSTStop respectively). For the positive control groups, mice were immunized with purified GAS M cloned from either GAS SF370 or GAS DSM 2071 strains (groups indicated as 192SF and 192DSM respectively).

[299] Pooled sera from each group was collected before the first immunization and two weeks after the last one. Mice were infected with GAS about a week after.

[300] Immunized mice were infected using GAS strain 2071, a different strain from that used for the cloning of the selected proteins. (German Collection of Microorganisms and Cell Cultures, DSMZ).

[301] For infection experiments, DSM 2071 was grown at 37° C in THY broth until the OD_{600} was 0.4. Bacteria were pelletted by centrifugation, washed once with PBS, suspended, and diluted with PBS to obtain the appropriate concentration of bacteria/ml and administered to mice by intraperitoneal injection. Between 50 and 100 bacteria were

given to each mouse, as determined by plating aliquots of the bacterial suspension on 5 THY plates. Animals were observed daily and checked for survival. The results obtained after intraperitoneal challenge are shown in FIG. 22 and Table 6A. The results obtained after intranasal challenge are shown in Table 6B (note the increased survival rate).

EXAMPLE 6

[302] Using the model described above, selected GAS antigens were tested and some of them showed statistically significant protection rates (FIG. 14). Of these, GAS40 appeared to be particularly promising, giving protection efficacy above 50% against mouse challenge with a heterologous GAS strain.

EXAMPLE 7

FACS analysis

[303] Bacteria were grown in THY to OD_{600} = 0.4, washed twice with PBS, suspended in NCS (Newborn Calf Serum, Sigma), incubated for 20 min at RT, and dispensed in a 96-well plate (20 µl/well). Eighty µl of preimmune or immune mouse sera, diluted in PBS-0.1% BSA, were added to the bacterial suspension to a final dilution of 1:200 and incubation was performed on ice for 30 min. After washing twice with PBS-0.1%BSA, bacteria were incubated on ice for 30 min in 10 µl of Goat Anti-Mouse IgG, $F(ab')_2$ fragment-specific-R-Phycoerythrin-conjugated (Jackson Immunoresearch Laboratories Inc.) in PBS-0.1% BSA-20% NCS to a final dilution of 1:100.

[304] Following incubation, bacteria were washed with PBS-0.1% BSA, suspended in 200 µl PBS and analyzed using a FACS Calibur cytometer (Becton Dikinson, Mountain View, CA USA) and Cell Quest Software (Becton Dikinson, Mountain View, CA USA). The results are shown in FIG. 17.

EXAMPLE 8

Distribution of GAS40 on the bacterial surface

Immunogold labeling and electron microscopy

[305] GAS were grown in THYE medium to mid-log phase, washed and resuspended in PBS. Formvar-carbon-coated nickel grids were floated on drops of GAS suspensions for 5 min, fixed in 2% PFA for 5 min, and placed in blocking solution (PBS containing 1% normal rabbit serum and 1% BSA) for 30 min. The grids were then floated on drops of primary antiserum diluted 1:20 in blocking solution for 30min at RT, washed, and floated on secondary antibody conjugated to 10 nm gold particles diluted 1:10 in 1% BSA for 30 min.

[306] The grids were washed with PBS then distilled water and air dried and examined using a TEM GEOL 1200EX II transmission electron microscope. Preimmune serum from the same animals were used as a negative control. The results are shown in FIG. 18.

EXAMPLE 9

Opsonophagocytosis and Bacterial Growth Inhibition

Preparation of bacterial inoculum

[307] Bacterial cells were grown in THY medium until they reached the middle exponential phase (OD600 0.4) at 37°C. Bacteria were washed twice in chilled saline solution and suspended in MEM medium with the volume being adjusted for each strain depending on the amount of bacteria used. Bacterial cells were kept in ice until used in the assay.

Preparation of peripheral mononuclear cells (PMN)

[308] PMN were prepared from buffy coats of heparinized blood from healthy volunteers. The buffy coat was incubated for 30 minutes in a solution containing dextran, NaCl and

Heparin (rate 1:1). After incubation the supernatant, enriched in leukocytes, was removed, transferred to a clean tube, and centrifuged at 700xg for 20 minutes. A short wash in water was performed to break red blood cells, and then a solution of NaCl was added to restore the appropriate salt concentration. After this step cells were centrifuged, washed, and suspended in MEM at a suitable concentration.

Opsonophagocytosis assay

[309] GAS strains were incubated with heat inactivated immune mice serum (or preimmune for the control), human PMN, and baby rabbit complement for 1 hour of incubation at 37°C. Samples taken immediately before and after the incubation were plated on THY blood agar plates. Phagocytosis was evaluated comparing the difference in the number of colonies at the two times for the preimmune and the immune serum. Data were reported as logarithm number of grown colonies at t=0 - logarithm number of grown colonies at t=60. See FIG. 20.

Bacterial growth inhibition

[310] Complete heparinized blood from mice immunized with GAS40 was incubated with bacterial cells grown as described above. Blood of mice immunized only with protein buffer was used as a control. The samples were rotated end over end for 3 hours at 37°C. Reactions were plated on THY blood agar plates, and CFU were counted. Growth inhibition was evaluated by comparing the number of colonies in the samples and in the control. See FIG. 19.

EXAMPLE 10

Expression of GAS40

[311] To test whether the GAS40 gene, which appears to be well conserved, was actually expressed in different strains, total cell extracts from a panel of distinct GAS strains were loaded on SDS-PAGE and probed with immune sera raised against the recombinant

GAS40. As shown in FIG. 16, the protein is expressed at a detectable level in all the strains tested, although a certain level of variability is observed.

[312] Cell extracts were obtained by growing the cells at 37°C to OD_{600} 0.32 in 10 ml of THY. The cell pellet was washed in PBS, resuspended in lysis buffer (40% sucrose, 0.1 M KPO_4 pH 6.2, $MgCl_2$ 10 mM, and Roche's COMPLETE™ EDTA-free), and digested for 3 hours with 400U of mutanolysin and 2 mg/ml lysozyme. The insoluble fraction was separated by centrifugation and the supernatant was analyzed.

EXAMPLE 11

Cloning of GAS40 domains

[313] Computational structural studies based on the amino acid sequence of GAS40 identified two potential coiled coil regions, one at the C-terminus and one at the N-terminus (see also WO 05/032582). This prediction was used to clone and express two isolated protein domains, one of predicted 305 (40N) amino acids and one of 568 amino acids (40C). Two sets of primers (see below) were used to amplify the two distinct coding regions by PCR, each containing one of the predicted coiled-coil domains:

40N-F: 5′-GTGCGTCATATGCAAGTCAAAGCAGATGATA-3′ (SEQ ID NO:965)

40N-R: 5′-ACTCGCTAGCGGCCGCTTGGTATTGATTTAATTGATTAC-3′(SEQ ID NO:966)

40C-F: 5′-GTGCGTCATATGGATATTCCAGCAGATCGTA-3′ (SEQ ID NO:967)

40C-R:5′-ACTCGCTAGCGGCCGCGACTCCTGCTTTAAGAGCT-3′ (SEQ ID NO:968)

[314] The DNA fragments were then cloned into pET21b+ and pGEX vectors and expressed in *E. coli*. Only the N-terminal domain gave a product of the expected size (40N). See FIG. 21. GAS40N consists of a 292 amino acid portion of GAS40 with a methionine at the start of the sequence and a polyhistidine tail at the end.

EXAMPLE 12

GAS40 is surface-exposed across different M strains

[315] FIG. 23 demonstrates that GAS40 is surface exposed across different M strains. The data were obtained using convalescent sera from patients with or recovered from a GAS infection.

EXAMPLE 13

GAS40N does not react with four anti-GAS40 monoclonal antibodies

[316] FIG. 24 demonstrates that four different monoclonal antibodies against GAS40 (28A8, 29G2, 2E4 and 4E6) do not react with the GAS40N part of the molecule.

[317] Note the blank vertical gel under the heading "GAS40N" and the reactivity with the GAS40 protein (see "GAS40" column). These results indicate that these GAS40 monoclonal antibodies were not raised against a GAS40N-specific epitope.

[318] The FACS graphs shown in FIG. 24 demonstrate that the four GAS40 monoclonal antibodies do not appear to bind to any surface exposed molecules on the M23 strain (no shift in graph peaks), whereas at least three of the four GAS40 monoclonal antibodies appear to bind to surface exposed molecules on the M4 strain. This result may be explained by the fact that the M23 is a capsulated strain and so the capsule is blocking the surface exposed GAS40 molecules.

EXAMPLE 13

Surfome analysis: Identification of GAS surface proteins using protease digestion, liquid chromatography, and tandem mass spectrometry

[319] Bacterial strains and culture. *Streptococcus pyogenes* bacteria from the hypocapsulated M1 wild-type strain SF370 (M1 serotype) (Ferretti *et al.*, Proc. Natl. Acad. Sci. U.S.A.

98, 4658-63, 2001), CDC SS-90 (M3 serotype), and 2071 (M23 serotype) were grown in Todd-Hewitt broth (THB) at 37°C and 5% CO_2, until an OD_{600} = 0.4 was reached (exponential growth phase). After culture, bacteria were harvested by centrifugation at 3,500 × g for 10 minutes at 4 °C, washed three times with phosphate-buffered saline (PBS), and used in the experiments described below. Genomic data for M1 are available at the TIGR website (URL address: http file type, www host server, domain name tigr.org), together with bioinformatics prediction results on function.

[320] Bacterial surface digestion. Protease digestion of the bacterial surface was carried out separately with two different enzymes: Sequencing Grade Modified Trypsin (Promega, Madison, WI) and proteinase K (Promega). Cells from a 100 ml initial culture were resuspended in 0.8 ml phosphate-based buffer (2.7 mM KCl, 1.5 mM KH2PO4, 13.7 mM NaCl, 8.1 mM Na2HPO4) containing 40% sucrose.

[321] Digestions were carried out in the presence of 5 mM DTT for 30 minutes at 37°C with either 20 μg trypsin (ph 7.4) or 10 μg proteinase K (pH 6.0). The digestion mixtures were centrifuged at 3,500 × g for 10 minutes at 4°C. The supernatants containing the digested peptides were filtered through a Millipore filter with a pore size of 0.22 μm. Protease reactions were stopped by adding 10% v/v of 1% formic acid. Prior to analysis, salts in samples were removed by off-line HPLC, with a 7 min gradient of 2-80% acetonitrile (ACN) in 0.1% formic acid. Fractions collected were pooled and concentrated with a Speed-vac. Supernatants were kept at -20 °C until further analysis.

[322] Multidimensional protein identification technology (MudPIT). Two different platforms were used for the chromatographic separation of peptides and further identification by tandem mass spectrometry (MS/MS).

[323] In the first platform, peptides were separated by two-dimensional nano-liquid chromatography (2-D LC), spotted directly onto a MALDI target, and analyzed by MALDI TOF-TOF (off-line coupled 2-D LC/MALDI MS/MS). The chromatographic system (Dionex, Amsterdam, The Netherlands) consisted of a FAMOS autosampler, an

UltiMate micropump with UV detector and a Switchos column-switching device, as described in (Mitulovic *et al.*, Proteomics. 2004 Sep;4(9):2545-57). The UltiMate pump was set to operate at a flow rate 300 nL/min. The flow of the Switchos loading pump, which was used to carry the sample from the sample loop to the first column, was set to operate at 30 μL/min.

[324] Briefly, peptide separation was performed as follows. In the first dimension, peptides were loaded on a strong cation exchange (SCX) column (10 cm × 320 μm i. d.) and eluted isocratically by applying 5 increasing NaCl concentrations (0.01, 0.05, 0.1, 0.5 and 1 M). In the second dimension, peptides were separated by a reversed phase C18 analytical column (15 cm × 75 μm i. d., C18 PEPMAP100™, 3 μm, 100 Å) through a C18 trap column (PEPMAP™ C18 μ-precolumn, 300 μm i.d. × 1 mm, Dionex). Peptides were eluted with a 45-min gradient from 5 to 50% of 80% ACN in 0.1% formic acid at a flow rate of 300 nl/min.

[325] Eluates were continuously spotted onto an ANCHOR-CHIP® MALDI target (Bruker Daltoniks, Bremen, Germany) every 60 s using a Proteineer FC robot (Bruker Daltoniks) prepared with a thin layer of a saturated solution of α-cyano-4-hydroxycynnamic acid in acetone, every 60 s using a Proteineer FC robot (Bruker Daltoniks). Prior to spotting, the target was prepared with a thin layer of a saturated solution of α-cyano-4-hydroxycynnamic acid in acetone. After fraction collection, every spot was manually recrystallized with 0.6 μl of ethanol/acetone/0.1% trifluoroacetic acid (6:3:1).

[326] Mass spectrometry analysis was performed automatically with an Ultraflex MALDI TOF-TOF instrument, under the control of the WARP LC software (Bruker Daltoniks). First, MS spectra of all the spotted fractions were acquired for peak selection and further MS/MS spectra acquisition of selected peaks. Searching and identification of peptides were performed in batch mode with a licensed version of MASCOT in a local database. The MASCOT search parameters were: (1) species: *S. pyogenes* strain SF370; (2) allowed number of missed cleavages (only for trypsin digestion): 6; (3) variable post-

translational modifications: methionine oxidation; (4) peptide tolerance: ±300 ppm; (5) MS/MS tolerance: ±1.5 Da and (6): peptide charge: +1.

[327] In the second platform, peptides were separated by nanoLC-MS/MS on a CapLC HPLC system (Waters, Milford, MA, USA) connected to a Q-ToF Micro ESI mass spectrometer equipped with a nanospray source (Waters, Milford, MA, USA). Samples were resuspended in 5% (v/v) ACN, 0.1% (v/v) formic acid (Solvent A) and loaded on a C18 trap column (300μm i.d. x 5 mm, LC Packings, Amsterdam, The Netherlands). After 3 min, the flow was switched to an Atlantis C18 NanoEase column (100μm i.d. x 100mm, Waters, Milford, MA, USA) and a solvent gradient was started. The applied flow rate was of 4μL/min and a flow splitter was set up to direct a nanoflow of 400 nL/min through the analytical column.

[328] Peptides were eluted applying a linear gradient in 50 min from 2% to 60% Solvent B (95% (v/v) ACN, 0.1% (v/v) formic acid). The eluted peptides were subjected to an automated data-dependent acquisition program, using the MassLynx software (Waters, Milford, MA, USA), where a MS survey scan was used to automatically select multi-charged peptides to be subjected to MS/MS fragmentation. Up to three different components where subjected to MS/MS fragmentation at the same time. For all the samples a second nanoLC-MS/MS analysis was carried out for the selective fragmentation of mono-charged peptide species.

[329] All the acquired MS/MS spectra were converted in PKL file format and protein identification was achieved by database searching using licensed version of MASCOT running on local database. The applied searching criteria were the following: peptide tolerance ±500ppm, MS/MS tolerance ±0.3Da, missed cleavage 6, peptide charge states from 1+ to 4+.

[330] Cloning, expression and purification of recombinant proteins, and preparation of preimmune and immune sera, was done as described in Maione *et al.*, Science 309, 148-50, 2005.

[331] FACS analysis. FACS analysis was performed as follows. About 105 bacteria were washed with 200 µl of PBS, centrifuged for 10 minutes at 3,500 × g, at 4°C, and then resuspended in 20 µl of PBS-0.1% BSA. Eighty µl of either pre-immune or immune mouse serum diluted in PBS-0.1% BSA were added to the bacterial suspension to a final dilution of either 1:100, 1:250, or 1:500, and incubated on ice for 30 min.

[332] Bacteria were washed once by adding 100 µl of PBS-0.1% BSA, centrifuged for 10 minutes at 3,500 × g, at 4°C, resuspended in 200 µl of PBS-0.1% BSA, and centrifuged again. The bacteria were resuspended in 10 µl of phycoerythrin-conjugated goat anti-mouse IgG $F(ab')_2$ fragment (Jackson Immunoresearch Laboratories Inc.) in PBS-0.1% BSA to a final dilution of 1:100, and incubated on ice for 30 minutes in the dark. Bacteria were then washed by adding 180 µl of PBS-0.1% BSA, and centrifuged for 10 minutes at 3,500 × g, at 4°C, and resuspended in 200 µl of PBS.

[333] The bacterial resuspension was then analyzed using a FACS Calibur instrument (Becton Dickinson, Mountain View, CA USA), and 10,000 events were acquired. Data were analyzed using Cell Quest Software (Becton Dickinson) by drawing a morphological dot plot (using forward and side scatter parameters) on bacterial signals. A histogram plot was then created on FL2 intensity of fluorescence log scale.

EXAMPLE 14

Surfome of SF370 (M1 serotype)

[334] The approach described above unambiguously identified 72 proteins from a total of 177 tryptic peptides and 107 peptides generated by proteinase K digestion. Ten proteins were identified from proteinase K-derived peptides, and 19 proteins were identified from both trypsin and proteinase K peptides. Table 9 shows the list of the proteins identified by applying both LC/MS/MS platforms, based on ESI-q-TOF and MALDI-TOF/TOF technologies. The protein list is the result of joining data from three independent surface digestion experiments for each protease. At least two chromatographic runs, followed by

MS/MS analysis, were performed per sample and platform. Approximately 5 pmol of peptides were loaded into both 2-D LC/MALDI MS/MS and LC/ESI MS/MS systems. Assignment of subcellular location and specific protein features was made by means of PSORT software.

[335] By scanning the sequence of each identified protein for the presence of signatures indicative of specific cellular localization, the 72 proteins could be grouped into 4 major families: the cell wall-anchored protein family containing LPXTG (SEQ ID NO:931)-like motifs (12 proteins), the lipoprotein family (11 proteins), the transmembrane protein family carrying one or more transmembrane spanning regions (37 proteins) and the family of secreted proteins (8 proteins). Based on genome computer analysis, the total number of GAS SF370 proteins which could be attributed to each of these protein families are 17, 28, 489 and 67, respectively. Therefore, while a large proportion of all predicted cell-wall anchored proteins and lipoproteins were identified, less than 7% of secreted proteins and membrane spanning proteins were found exposed on the cell surface.

[336] This discrepancy was expected for the secreted proteins, being consistent with the notion that most of them are released out of the cell and only a fraction remain partially associated to the cell wall. On the contrary, the small number of identified transmembrane proteins was somehow surprising and suggested that a large fraction of these proteins are either deeply embedded in the membrane, or poorly expressed or both. Interestingly, only 4 PSORT-predicted cytoplasmic proteins were found associated to the external side of the cell (Table 9), and all of them belong to the category of cytoplasmic proteins reported to be membrane-associated in most, if not all, bacteria so far analyzed. They included the elongation factor Tu, reported to be membrane-associated in other bacteria (Marques *et al.*, Infect. Immun. 66, 2625-31, 1998; Dallo *et al.*, Mol. Microbiol. 46, 1041-51, 2002), two ribosomal proteins (Spence & Clark, Infect. Immun. 68, 5002-10, 2000; Kurar & Splitter, Vaccine 15, 1851-57, 1997), and a hypothetical protein possibly involved in cell wall localization and side chain formation.

[337] See also Tables 7 and 8 and FIGS. 28-104. Although the mass spectrometry technique is not quantitative, the M protein seemed to be the most abundant; 14 MS/MS spectra of M protein peptides were identified after trypsin digestion.

[338] Most of the proteins (60) were identified from peptides generated by trypsin digestion. Proteinase K released peptides corresponding to 30 proteins; 19 proteins were identified by both enzymes. Proteinase K was especially useful for the recovery of peptides corresponding to membrane proteins with a high number of transmembrane domains (TMD): *e.g.*, proteins NT01SP0454, NT01SP0906 and NT01SP1664, with 7, 10 and 4 TMD, respectively, which were not identified from trypsin digestion. The number of identified peptides per protein ranged from one to several tens.

[339] FIGS. 25A-B and 26A-B show the high coverage obtained after digestion with both proteases of the cell-wall protein NT01SP1652 (GAS190; SEQ ID NO:117). GAS190 contains the anchoring sequence LPXTG (SEQ ID NO:931). It has been previously described as "OrfX" and belonging to the vir regulon, which organizes the expression of several bacterial virulence factors under the control of the Mga regulator. The function of GAS190 is, so far, unknown, although it may be a fibronectin-binding protein. The wall-associated region can cover from about 50 to as many as 125 amino acid residues.

[340] FIGS. 25A (SEQ ID NOS:932-949) and 25B show the coverage of the GAS190 protein sequence (50.6%) by the 35 proteinase K-generated peptides. The zone lacking coverage by tryptic peptides (shown in FIG. 25B between arrows) was widely represented under proteinase K digestion, although a high degree of redundant information was obtained. Most of the peptides identified corresponded to the most exposed region of the protein, *i.e.*, the first half of the sequence from the N-terminus.

[341] The 11 tryptic-generated peptides and their alignment along the GAS190 protein sequence are shown in FIGS. 26A (SEQ ID NOS:950-961) and 26B, resulting in a coverage of 34.6%. Peptides from the trypsin digestion were found as close as 13 amino acid residues to the LPXTG (SEQ ID NO:931) in the GAS190 protein. Note the absence

of sites for trypsin digestion (K/R) between the two arrows in FIG. 2B, which makes the generation of tryptic species in that zone theoretically impossible. Only one peptide lacking K or R before its N-terminus was identified (VDGIPPISLTQK, SEQ ID NO:969), which corresponds to the actual N-terminus of the mature form of the protein according to PSORT predictions. Peptides having more than one trypsin-missed cleavage site (6 out of 11) were relatively abundant. For example, the peptide IKTAPDKDKLLFTYHSEYMTAVK (SEQ ID NO:970) contains 3 internal lysines that were not C-cleaved by trypsin; for some peptides of M protein, up to 6 cleavage sites were missed.

[342] Not all the proteins identified were as extensively covered by their respective generated peptides. Proteins containing cell wall-anchoring motifs showed the highest degree of coverage. Proteins with at least one predicted TMD (according to PSORT prediction) were identified, in general, from a low number of peptides (Table 9).

EXAMPLE 15

Identification of GAS surface proteins after overproduction of membrane-delimited structures after antibiotic treatment (SF370 serotype)

[343] Bacterial culture and antibiotic treatment. *Streptococcus pyogenes* SF370 cells were grown in THB at 37 °C and 5% CO_2, until an OD_{600} = 0.4 was reached (exponential growth phase). Growth medium was harvested after centrifugation at 3,500 × g for 10 minutes at 4 °C. Bacterial suspension was diluted twofold by adding THB containing antibiotics (0.7 μg/ml penicillin; 10 μg/ml vancomycin, final concentration) and the culture was left for 80 min. FIG. 107 is an electron micrograph showing membrane-delimited structures produced upon penicillin treatment.

[344] Recovery of membrane-delimited structures. Supernatant was filtered (0.22 μm) and membrane-delimited structures were recovered by ultracentrifuge at 200,000 × g for 90 minutes at 4 °C. The pellet was then washed once in PBS and then resuspended in the same buffer.

[345] Proteomic analysis of membrane-delimited structures. Ultracentrifugation pellets were subjected to SDS-PAGE. The bands thus separated were picked, destained, trypsin-digested, desalted by using Zip-Tips (Millipore), and analyzed by MALDI-TOF using an Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker Daltoniks). MASCOT software was used for spectra analysis and identification.

[346] Proteins identified by this method are shown in Table 8. The majority of the identified proteins are surface-exposed proteins (secreted, membrane-bound, or lipoproteins). No cell wall proteins were observed either in this fraction or in the membrane-delimited structures. Thus, most of the protein content of the membrane-delimited structures is of potential interest for vaccine development.

EXAMPLE 16

Identification of GAS surface proteins after chemical fractionation (SF370 serotype)

[347] Bacterial pellet preparation. Bacteria (GAS SF370) were grown in 250 ml THB at 37°C and 5% CO2 until an OD600=0.4 was reached (exponential growth phase). After culture, bacteria were harvested by centrifugation at 3,500 x g for 10 minutes at 4°C and washed with phosphate-buffered saline (PBS). Bacteria were re-suspended in 20 ml of 6M guanidinium (urea or SDS also could be substituted for guanidinium), 200 mM Tris HCl and disrupted with 3 cycles at 1.7 kBar in a Basic Z 0.75V Model Cell Disrupter equipped with an "one shot head" (Constant System Ltd, Northants, England). The lysate was then centrifuged at 20,000 x g for 20 minutes at 4°C. The resulting supernatant was filtered through a 0.22 μm membrane and spun in an ultracentrifuge at 200,000 x g for 2 hours at 4°C. The pellet was washed with PBS (200,000 x g for 30 minutes at 4°C).

[348] Total digestion. Three hundred microliters containing 10 μg of trypsin in 50 mM ammonium bicarbonate, 5 mM DTT was added to the pellet. Digestion was allowed to proceed overnight at 37°C.

[349] Separation by SDS-PAGE. Three hundred microliters containing 9 μg of mutanolysin in 50 mM Tris-HCl pH7.5 was added to the pellet. Digestion was allowed to proceed 3 hours at 37°C, which permits the proteins to enter the gel. Proteins were separated by electrophoresis in a 12 % polyacrylamide gel.

[350] In-gel protein digestion and sample preparation for mass spectrometry analysis. Protein spots bands were excised from the gels, washed with 100 mM ammonium bicarbonate/acetonitrile 50/50 (V/V), and dried using a SpeedVac centrifuge (Savant, Holbrook, NY). Dried spots were digested 2 hours at 37°C in 12 μl of 0.012 μg/μl sequencing grade modified trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate. After digestion, 5 μl of 0.1 % trifluoacetic acid was added, and the peptides were desalted and concentrated with ZIP-TIPs (C18, Millipore).

[351] Peptides were eluted with 2 μl of 5 g/l 2,5-dihydroxybenzoic acid in 50% acetonitrile/0.1% trifluoroacetic acid onto the mass spectrometer Anchorchip 384 (400 μm, Bruker Daltonics, Bremen, Germany), allowed to air dry at room temperature, and analysed by matrix-assisted matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Mass spectra were collected on a Bruker UltraFlex mass spectrometer, calibrated using a peptide calibration standard (1000-4000 Da) from Bruker (part no206195). Peptide masses were determined using FlexAnalysis (Version 2.2, Bruker). Protein spot identifications were carried out by both automatic and manual comparison of experimentally generated monoisotopic values of peptides in the mass range of 1000-3500 Da with computer-generated fingerprints using Mascot software.

[352] Results. Fragmentation of peptide of m/z 1372.7, 1202.6 identified the C5A peptidase precursor (15675796). Fragmentation of peptides of m/z 1706.8, 1880.1 identified the M protein type 1 (15675799).

EXAMPLE 17

Surfome analysis of the highly capsulated strain M3

[353] GAS is surrounded by a hyaluronic acid-based capsule whose thickness can vary from strain to strain. Capsule plays an important role in bacterial virulence, and in general the highly capsulated strains are the most virulent strains. It is expected that the number of proteins with accessible external domains will depend on the thickness of the surrounding capsule. To verify that, we characterized the surfome of the highly capsulated strain M3.

[354] The strain was grown as described above and the capsule content was determined by measuring the amount of hyaluronic acid recovered as described below. Under these conditions, M3 produced approximately 51 fg of hyaluronic acid per cfu, three times as much as SF370 strain. See Table 10 and FIG. 108. M3 bacteria were then subjected to the same surfome analysis described above.

[355] As shown in Table 11, only 10 proteins could be detected upon proteolytic digestion and mass spectrometry analysis; all but one (Elongation Factor Tu) were predicted to be surface-associated. They include 5 LPXTG (SEQ ID NO:931)-carrying proteins, two membrane proteins and two secreted proteins. Interestingly, 5 of these proteins belong to the hypothetical/unknown protein family. Furthermore, with the exception of the F2-like protein, whose coding gene is absent in SF370, and of the putative penicillin binding protein and the hypothetical protein SPs1270, all the other proteins also belong to the SF370 surfome.

[356] In conclusion, the presence of capsule does interfere with surface accessibility of proteins, as judged by the reduced number of peptides which could be generated upon proteolytic digestion of whole cells.

EXAMPLE 18

Surfome validation

[357] The almost complete absence of cytoplasmic proteins in both surfomes suggested that our procedure was selective for surface-exposed protein identification. To further confirm the robustness of the procedure we carried out two types of analysis.

[358] First, we subjected the 37 trans-membrane proteins of SF370 surfome to topological prediction using PSORT (see URL address http file type, www host server, domain name nibb.ac.ip, form directory) and asked whether the peptides generated by the proteolytic cleavage and identified by MS/MS were located, as it would be expected in the case of a correct topological prediction, within the external domains. As shown in Figure 106, for 26 out of 37 membrane proteins in silico analysis and experimental MS/MS data were perfectly consistent. On the contrary, the corresponding identified peptides of the remaining 11 proteins mapped on PSORT-predicted intracellular domains.

[359] This contradiction prompted us to manually inspect the topological and functional annotations of each of these 11 proteins. We concluded that it was desirable to revisit the predicted trans-membrane organization of at least 6 out of 11 proteins. In particular, the two peptides derived from the putative cell division protein NT01SP0014, homologous to the FtsH protein family, are located within a well conserved protein domain known to be extracellular in FtsH proteins (Tomoyasu *et al.*, J. Bacteriol. 175, 1352-57, 1993; Akiyama *et al.*, J. Biol. chem. 271, 22326-33, 1998; Amara *et al.*, Microbiol. 144, 1197-1203, 1998) was found to carry a well conserved protein domain known to be extracellular in these proteins.

[360] The four peptides of NT01SP1255, a second putative cell division protein homologous to FtsZ, are located within the C-terminal part of the molecule, a region that in Bartonella bacilliformis was found immunogenic and surface-exposed (Padmalayam *et al.*, J. Bacteriol. 179, 4545-52, 1997).

[361] The two hypothetical proteins NT01SP0289 and NT01SP1789 carry transmembrane regions (TMR) with a very poor PSORT score and, having a leader peptide cleavage sites next to a Cys residue, are likely to be lipoproteins rather than integral membrane proteins

[362] NT01SP0947 has two TMRs the second of which with a very poor PSORT score (-0.32 as opposed to –8.12 of the first TMR). If the second TMR were in fact not real, the C-terminal part of the molecule, which carries a typical sortase domain, would be exposed on the surface, which would be consistent with the sortases mechanism of action (Paterson & Mitchell, Trends Microbiol. 12, 89-95, 2004).

[363] Finally, NT01SP0154, a putative glycine-betaine binding permease protein, is predicted to have 6 TMRs, one of which with a poor score. Again, if the weak TMR is neglected, the topological organization would change and the C-terminal region, where the two MS/MS identified peptides fall, would become surface-exposed. Indeed, polyclonal antibodies against the C-terminal domain of the protein efficiently bound GAS SF370 whole cells when tested by FACS.

[364] The second type of study we used to validate our surfome analysis was based on FACS analysis using protein-specific antibodies. Polyclonal antibodies were produced against 51 recombinant proteins selected among the SF370 surfome, and the antibodies were tested for their capacity to bind to whole bacteria. All but 7 sera were positive in the assay, indicating that each corresponding protein was sufficiently exposed on the bacterial surface to be accessible to antibody binding (see also Table 9). Similarly, polyclonal antibodies against 4 of the 10 proteins belonging to the M3 surfome were tested by FACS and three of them were capable of binding to M3 cells (See FIG. 105; Table 9).

[365] From these data we concluded that our approach for surfome analysis is accurate in determining which proteins are entirely or partially exposed on bacterial cell surface.

EXAMPLE 19

Application of surfome analysis to vaccine discovery

[366] From previous experience with Meningococcus B and Group B Streptococcus we know that for an antigen to be a vaccine candidate it is desirable that it be well expressed and exposed on the surface of the bacterial cell (Pizza *et al.*, Science 287, 1816-20, 2000; Maione *et al.*, Science. 2005 Jul 1;309(5731):148-50.). Because surfomes, by definition, include this category of proteins, surfome analysis is an ideal approach to identification of new vaccine candidates. In support of this is the observation that 6 of the 10 reported GAS protective antigens whose genes are present in SF370 are part of the SF370 surfome. See Table 12 and references cited therein.

[367] Because several of the SF370 surfome proteins have never been tested for protection, we investigated whether some of them could elicit protective responses in the mouse model. Unfortunately, SF370 is not virulent in the mouse, the LD50 dose being over 108 CFUs. Because we expect the GAS surfome to vary somewhat from strain to strain depending upon protein expression level, capsule thickness, and gene variability, before testing the SF370 surfome proteins in protection studies against a different strain, we investigated which of these proteins were also exposed on the surface of the challenge strain.

[368] To this end, we defined the surfome of M23 DSM2071, one of the GAS strains we routinely used for mouse challenge. Because the genome sequence of M23 DSM2071 is not available, only the exposed proteins that are in common to SF370 or to the other six GAS strains whose sequences are available in the public databases (URL address: http file type, www host server, domain names tigr.org and ncbi.nlm.nih.gov) are identified, whereas the M23 DSM2071-specific proteins remain uncharacterized.

[369] As shown in Table 7, a total of 17 proteins were unambiguously identified: 5 cell wall anchored proteins, 4 lipoproteins, 5 membrane proteins, 2 secreted proteins and 1 cytoplasmic protein. All these proteins have an analogue in SF370 and all but two (the

putative zinc-containing alcohol dehydrogenase and the putative, RofA-related, regulatory protein) were also included in the SF370 surfome (Table 9). Interestingly, most (13 out of 17) of the identified proteins belong to the family of putative/hypothetical proteins.

[370] Of the 17 proteins belonging to the M23 DSM2071 surfome, 14 proteins were successfully expressed in *E. coli* as either soluble His-fusions or GST-fusions (Table 7). Proteins NT01SP0908 and NT01SP0485 were not considered for expression because they have significant homology with human proteins. Five-week-old female CD 1 mice (10 mice/group) were immunized with 20 μg recombinant protein administered intraperitoneally (i.p.) at 0, 21 and 35 days with complete Freund's adjuvant (CFA) the first time and with incomplete Freund's adjuvant (IFA) the two following times. Blood samples were collected before the first and after the third immunizations. Immunized mice were challenged intranasally (i.n.) with 106 colony forming units (CFUs) of DSM 2071 strain grown in THY broth at OD_{600} = 0.4. CFU titer of the infecting dose was verified by plating on 5 THY/blood plates. Mice were monitored daily, and the final survival rate was calculated after 10 days.

[371] As shown in Table 14, two proteins were protective in this model: the M protein (90% survival rate) and NT01SP0336 (70% survival rate). NT01SP0336, which corresponds to GAS57 in the SF370 serotype, is a putative cell envelope proteinase carrying a typical cell anchoring LPXTG (SEQ ID NO:931) motif.

[372] Interestingly, GAS57 provided little or no protection in a mouse model in which intraperitoneal immunization was followed by intraperitoneal challenge. GAS40 was protective in both models, even though the survival rate in the intranasal challenge model was higher.

EXAMPLE 20

Capsule hyaluronic acid content determination

[373] Cells from a 10-ml exponential-phase culture (OD600 = 0.4) are washed twice with water and then resuspended in 500 µl of water. Capsule is released by shaking with 1 ml of clorophorm. After clarifying the sample by centrifugation, the hyaluronic acid content of 50 µl of the aqueous phase is determined by measuring absorbance at 640 nm after adding to the sample 1 ml of a solution containing 20 mg of Stains-All product (Sigma Chemical Co.) and 60 µl of glacial acetic acid in 100 ml of 50% formamide. Absorbance values are compared with a standard curve generated using known concentrations of hyaluronic acid.

EXAMPLE 21

Identification of surface exposed domains of GAS antigens

[374] In silico prediction algorithms initially identified 684 genes encoding for products likely to be secreted or associated with the bacterial surface. See Pizza *et al.*, Science 287, 1816-20, 2000; Tettelin *et al.*, Proc. Natl. Acad. Sci. U.S.A. 99, 12391-96, 2002. Of these, 207 were predicted to contain more than two transmembrane spanning regions. The protein sequences were searched for isolated domains of at least 50 amino acids which were predicted to lay on the surface of the cell (*e.g.*, extracellular loops, amino-terminal, or carboxy-terminal domains). Surface exposure was assessed with the aid of the on–line web server TMPRED (see URL address: http file type, www host server, domain name.ch.embnet.org, software/TMPRED_form.directory), which is able to predict membrane-spanning regions and their orientation using an algorithm based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins.

[375] Each of the identified domains was cloned in parallel in two vectors containing either sequences coding for 6 histidine residues. Recombinant products were successfully expressed and purified from *E. coli*.

EXAMPLE 22

Immunization with surface-exposed GAS antigens

[376] Groups of 10 or more CD1 female mice aged between 6 and 7 weeks were immunized with 20 μg of a recombinantly produced surface-exposed GAS antigen suspended in 100 μl of suitable solution. Mice of each group received 3 doses, at days 0, 21 and 45. Immunization was performed through intraperitoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and with Incomplete Freund's Adjuvant (IFA) for the following two doses. Negative and positive control groups were used in each immunization scheme.

[377] Mice in the negative control group were immunized with *E. coli* proteins eluted from the purification columns following processing of total bacterial extract from an *E. coli* strain containing either the pET21b or the pGEX-NNH vector (thus expressing GST only) without any cloned GAS ORF (indicated as HisStop or GSTStop, respectively).

[378] Mice in the positive control groups were immunized with purified GAS M cloned from either GAS SF370 or GAS DSM 2071 strains (groups indicated as 192SF and 192DSM respectively).

[379] Serum from each mouse was collected before the first immunization and two weeks after the last immunization. The sera of mice in each group were pooled. Mice were infected with one of GAS strains 2071 (M23), 3348 (M1), or 2728 (M12) about a week after the last immunization. For infection, GAS strains were grown at 37° C in THY broth until OD600 0.4. Bacteria were collected by centrifugation, washed once with PBS, suspended, and diluted with PBS to obtain the appropriate concentration of bacteria/ml

and administered to mice by intraperitoneal injection. Between 50 and 100 bacteria were given to each mouse, as determined by plating aliquots of the bacterial suspension on 5 THY plates. Animals were observed daily and checked for survival.

[380] The results are shown in Figures 109A-111C and summarized in Table 15. A delta mean of >80 indicates that the tested domain is surface-exposed.

[381] The results demonstrate that each of the tested domains -- GAS35, GAS414, GAS426, GAS433, GAS434, GAS437, GAS438, GAS439, GAS461, GAS465-2, GAS469, GAS472, GAS473, GAS475, GAS477, GAS478, GAS495, GAS538, GAS543, GAS553, GAS561, GAS576, GAS577-2, GAS587, GAS591, GAS593, GAS636, GAS643, GAS649, and GAS663 -- is exposed on the surface of at least one of the three GAS strains tested. Some of the tested domains show a variable delta mean across the strains used (M1, M23 and M12), possibly because of the different "visibility" of these domains due to capsule masking (for instance M23 is a highly encapsulated strain). Domains GAS35, GAS414, GAS437, GAS438, GAS461, GAS465-2, GAS469, GAS472, GAS473, GAS475, GAS478, GAS495, GAS538, GAS553, GAS561, GAS577-2, GAS591, GAS593, GAS636, GAS643, GAS649, and GAS663 are surface-exposed on the surface of at least two of the three GAS strains tested. Domains GAS472, GAS473, and GAS553 are surface-exposed on all three of the tested GAS strains.

EXAMPLE 23

Protein microarray experiments

[382] Protein chips allow for the identification of clinical immunogenic prevalence of pathogenic proteins in human sera. Using protein chips we tested serum samples from 6 healthy donors, two of which had had a recent documented pharyngitis associated to GAS infection (SC and TM, see FIG. 112).

[383] FIG. 112 shows a diagram where the prevalent immunoreactive antigens are mapped and clustered according to signal intensity and response frequency for each of the 6 donors.

Experimental procedure

[384] 112 GAS proteins (19 GST fusions, 91 His-tagged and 2 native proteins) were purified, diluted in PBS at a concentration of 0.5 mg/ml and dispensed (6 µl each) in 384 well polypropylene micro plates. Four replicates of the protein solutions were spotted on nitrocellulose coated Fast Slides chips (Schleier & Schuell) using the VERSARRAY CHIPWRITER™ Pro System (BIO-RAD) equipped with TeleChem quill pins (TeleChem International Sunnyvale, CA, USA). Following the first printing of each protein, the pins were washed 7 times (6 seconds each), subjected to sonication (1 second) and dried under vacuum (2 seconds). Each chip contains at least one immunoglobulin and two BSA (Cy3 and Cy5-labelled, Amersham Bioscences) standard curves. After each printing process, each slide was scanned to check the signals of the Cy3 and Cy5-labeled BSA curves.

[385] Slides were pre-incubated overnight at 4 C° in the dark with agitation in 3% Top Block (Fluka-BioChemiKa, Cat. n° 37766) and 0.1% TPBS (0.1% Tween 20 in PBS). Slides were then incubated with human sera (1:1000 final dilution) for 1 hr at room temperature in the dark and were then washed 3 times (5 minutes each time) in 0.1% TPBS. Cy3 or Cy5 anti-human IgG (1:800), IgA or IgM (1:1000) were added and incubation was prolonged for 1 hr at room temperature in the dark.

[386] Slides were washed two times with TPBS (5 minutes each time), once with PBS (10 minutes), once with milliQ sterile water (30 seconds) and were then dried either at 37 C° for 10-20 minutes in the dark or using a nitrogen stream.

[387] Fluorescence signals were detected with a ScanArray 5000 Unit (Packard, Billerica, MA, USA) at high resolution (10 µm pixel size) and quantified with the ImaGene 6.0 software (Biodiscovery Inc, CA, USA)

[388] Elaboration of the collected data was performed using software which normalizes the data by interpolating the least-mean squares of the Ig controls to a sigmoid curve. The titer value corresponding to each experimental intensity signal is then referred to such sigmoid and normalized to a theoretic sigmoid curve extending over the whole dynamic range of the scanner.

Table 1. Sequence identifiers

GAS	annotation	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
4	full-length	1	650
5	full-length	2	651
6	full-length	3	652
10	full-length	4	653
15	full-length	5	654
16	full-length	6	655
18	full-length	7	656
22	full-length	8	657
23	full-length	9	658
24	full-length	10	659
25	full-length	11	660
29	full-length	12	661
30	full-length	13	662
35	full-length	14	663
36	full-length	15	664
39	full-length	16	665
40	strains SF370, 3280, 3348, 2913, 3789, 2580	17	666 (3280); 667 (3348); 668 (2913); 669 (3789); 670 (2580)
40	2634	18	675
40	2726	19	676
40	2721	20	677
40	3040, 3135	21	671 (3135); 678 (3040)
40	2722	22	679
40	2728	23	680
40	4883	24	681
40	2724	25	682
40	2894, 3650, 5529, 3776	26	672 (3650); 673 (5529); 674 (3776); 683 (2894);
40	2720	27	684
40	2725	28	685
40	4538	29	686
40	5531	30	687
40	5481	31	688
40	4959	32	689

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
40	DSM2071	33	690
40	4436	34	691
40	2727	35	692
40	2719	36	693
40	5455	37	694
40	5476	38	695
40	4088	39	696
40	MANFR10394	40	697
40	M8232	41	698
40	M315	42	699
40	SS1	43	700
41	full-length	44	701
42	full-length	45	702
45	full-length	46	703
49	full-length	47	704
54	full-length	48	705
56	full-length	49	706
57	full-length	50	707
58	full-length	51	708
60	full-length	52	709
62	full-length	53	710
63	full-length	54	711
64	full-length	55	712
65	full-length	56	713
67	full-length	57	714
68	full-length	58	715
69	full-length	59	716
70	full-length	60	717
72	full-length	61	718
74	full-length	62	719
75	full-length	63	720
76	full-length	64	721

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
77	full-length	65	722
78	full-length	66	723
81	full-length	67	724
82	full-length	68	725
83	full-length	69	726
84	full-length	70	727
85	full-length	71	728
86	full-length	72	729
87	full-length	73	730
88	full-length	74	731
89	full-length	75	732
91	full-length	76	733
92	full-length	77	734
93	full-length	78	735
94	full-length	79	736
95	full-length	80	737
96	full-length	81	738
97	full-length	82	739
98	full-length	83	740
99	full-length	84	741
100	full-length	85	742
101	full-length	86	743
102	full-length	87	744
103	full-length	88	745
104	full-length	89	746
105	full-length	90	747
108	full-length	91	748
117	full-length	92	749
123	full-length	93	750
130	full-length	94	751
131	full-length	95	752
137	full-length	96	753

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
142	full-length	97	754
143	full-length	98	755
149	full-length	99	756
152	full-length	100	757
157	full-length	101	758
158	full-length	102	759
159	full-length	103	760
160	full-length	104	761
163	full-length	105	762
165	full-length	106	763
166	full-length	107	764
168	full-length	108	765
171	full-length	109	766
175	full-length	110	767
177	full-length	111	768
178	full-length	112	769
179	full-length	113	770
183	full-length	114	771
187	full-length	115	772
188	full-length	116	773
190	full-length	117	774
191	full-length	118	775
192	full-length	119	776
193	full-length	120	777
194	full-length	121	778
195	full-length	122	779
198	full-length	123	780
201	full-length	124	781
202	full-length	125	782
205	full-length	126	783
206	full-length	127	784
207	full-length	128	785

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
208	full-length	129	786
210	full-length	130	787
217	full-length	131	788
218	full-length	132	789
219	full-length	133	790
220	full-length	134	791
224	full-length	135	792
236	full-length	136	793
242	full-length	137	794
249	full-length	138	795
251	full-length	139	796
253	full-length	140	797
259	full-length	141	798
262	full-length	142	799
264	full-length	143	800
268	full-length	144	801
271	full-length	145	802
277	full-length	146	803
282	full-length	147	804
284	full-length	148	805
286	full-length	149	806
290	full-length	150	807
291	full-length	151	808
292	full-length	152	809
294	full-length	153	810
299	full-length	154	811
309	full-length	155	812
327	full-length	156	813
366	full-length	157	814
372	full-length	158	815
380	full-length	159	816

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
382	full-length	160	817
362-1	full-length	161	818
384	full-length	162	819
389	full-length	163	820
396	full-length	164	821
405	full-length	165	822
406	full-length	166	823
414	full-length	167	824
421	full-length	168	825
425	full-length	169	826
426	full-length	170	827
428	full-length	171	828
433	full-length	172	829
434	full-length	173	830
437	full-length	174	831
438	full-length	175	832
439	full-length	176	833
457	full-length	177	834
460	full-length	178	835
461	full-length	179	836
465	full-length	180	837
469	full-length	181	838
472	full-length	182	839
473	full-length	183	840
474	full-length	184	841
475	full-length	185	842
477	full-length	186	843
478	full-length	187	844
486	full-length	188	845
492	full-length	189	846
493	full-length	190	847
494	full-length	191	848

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
495	full-length	192	849
500	full-length	193	850
504	full-length	194	851
509	full-length	195	852
511	full-length	196	853
527	full-length	197	854
529	full-length	198	855
533	full-length	199	856
535	full-length	200	857
538	full-length	201	858
540	full-length	202	859
543	full-length	203	860
545	full-length	204	861
553	full-length	205	862
558	full-length	206	863
560	full-length	207	864
561	full-length	208	865
564	full-length	209	866
565	full-length	210	867
574	full-length	211	868
576	full-length	212	869
577	full-length	213	870
579	full-length	214	871
586	full-length	215	872
587	full-length	216	873
591	full-length	217	874
592	full-length	218	875
607	full-length	219	876
609	full-length	220	877
625	full-length	221	878
626	full-length	222	879
636	full-length	223	880

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
640	full-length	224	881
643	full-length	225	882
645	full-length	226	883
649	full-length	227	884
650	full-length	228	885
653	full-length	229	886
657	full-length	230	887
663	full-length	231	888
685	full-length	232	889
117/40	full-length	233	890
40/117	full-length	234	891
40a-HIS	full-length	235	892
(place holder)		236	
40aRR-HIS	full-length	237	893
spy0047	full-length	238	894
spy0053	full-length	239	895
spy0056	full-length	240	896
spy0063	full-length	241	897
spy0069	full-length	242	898
spy0080a	full-length	243	899
spy0098	full-length	244	900
spy0127	full-length	245	901
spy0272	full-length	246	902
spy0461	full-length	247	903
spy0611	full-length	248	904
spy0666	full-length	249	905
spy0686	full-length	250	906
spy0688	full-length	251	907
spy0717	full-length	252	908
spy0792	full-length	253	909
spy080a	full-length	254	910
spy0913	full-length	255	911
spy1029	full-length	256	912

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
spy1073	full-length	257	913
spy1085	full-length	258	914
spy1200	full-length	259	915
spy1260	full-length	260	916
spy1281	full-length	261	917
spy1613	full-length	262	918
spy1721	full-length	263	919
spy1750	full-length	264	920
spy1805	full-length	265	921
spy1835	full-length	266	922
spy2005	full-length	267	923
spy2070	full-length	268	924
spy2092	full-length	269	925
spy2093	full-length	270	926
spy2178	full-length	271	927
g-21909751	full-length	272	928
NT01SP0246	full-length	273	929
M	full-length	274	
SagA	full-length	275	
Sfb1	full-length	276	
Shp	full-length	277	
linker	full-length	278	
linker	full-length	279	
linker	full-length	280	
40a-HIS	full-length	281	
4	fragment	282	
4	fragment	283	
5	fragment	284	
5	fragment	285	
15	fragment	286	
15	fragment	287	
16	fragment	288	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
23	fragment	289	
23	fragment	290	
24	fragment	291	
24	fragment	292	
24	fragment	293	
24	fragment	294	
24	fragment	295	
25	fragment	296	
25	fragment	297	
40	fragment	298	
54	fragment	299	
57	fragment	300	
57	fragment	301	
57	fragment	302	
63	fragment	303	
64	fragment	304	
64	fragment	305	
64	fragment	306	
64	fragment	307	
64	fragment	308	
64	fragment	309	
68	fragment	310	
72	fragment	311	
72	fragment	312	
84	fragment	313	
84	fragment	314	
84	fragment	315	
86	fragment	316	
86	fragment	317	
86	fragment	318	
86	fragment	319	
86	fragment	320	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
87	fragment	321	
89	fragment	322	
89	fragment	323	
89	fragment	324	
98	fragment	325	
98	fragment	326	
98	fragment	327	
98	fragment	328	
98	fragment	329	
102	fragment	330	
103	fragment	331	
108	fragment	332	
143	fragment	333	
143	fragment	334	
143	fragment	335	
143	fragment	336	
149	fragment	337	
152	fragment	338	
157	fragment	339	
157	fragment	340	
157	fragment	341	
157	fragment	342	
157	fragment	343	
157	fragment	344	
157	fragment	345	
157	fragment	346	
157	fragment	347	
157	fragment	348	
157	fragment	349	
157	fragment	350	
158	fragment	351	
163	fragment	352	
163	fragment	353	
163	fragment	354	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
163	fragment	355	
166	fragment	356	
166	fragment	357	
168	fragment	358	
177	fragment	359	
188	fragment	360	
188	fragment	361	
188	fragment	362	
188	fragment	363	
190	fragment	364	
190	fragment	365	
190	fragment	366	
190	fragment	367	
190	fragment	368	
190	fragment	369	
190	fragment	370	
190	fragment	371	
190	fragment	372	
190	fragment	373	
190	fragment	374	
190	fragment	375	
190	fragment	376	
190	fragment	377	
190	fragment	378	
190	fragment	379	
190	fragment	380	
190	fragment	381	
190	fragment	382	
190	fragment	383	
190	fragment	384	
190	fragment	385	
190	fragment	386	
190	fragment	387	
190	fragment	388	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
190	fragment	389	
190	fragment	390	
190	fragment	391	
190	fragment	392	
190	fragment	393	
190	fragment	394	
190	fragment	395	
190	fragment	396	
190	fragment	397	
190	fragment	398	
190	fragment	399	
190	fragment	400	
190	fragment	401	
190	fragment	402	
190	fragment	403	
190	fragment	404	
190	fragment	405	
190	fragment	406	
190	fragment	407	
190	fragment	408	
190	fragment	409	
190	fragment	410	
190	fragment	411	
191	fragment	412	
191	fragment	413	
191	fragment	414	
191	fragment	415	
191	fragment	416	
191	fragment	417	
191	fragment	418	
191	fragment	419	
191	fragment	420	
191	fragment	421	
191	fragment	422	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
191	fragment	423	
191	fragment	424	
191	fragment	425	
191	fragment	426	
191	fragment	427	
191	fragment	428	
191	fragment	429	
192	fragment	430	
192	fragment	431	
192	fragment	432	
192	fragment	433	
192	fragment	434	
192	fragment	435	
192	fragment	436	
192	fragment	437	
192	fragment	438	
192	fragment	439	
192	fragment	440	
192	fragment	441	
192	fragment	442	
192	fragment	443	
192	fragment	444	
192	fragment	445	
192	fragment	446	
192	fragment	447	
192	fragment	448	
192	fragment	449	
192	fragment	450	
192	fragment	451	
192	fragment	452	
192	fragment	453	
192	fragment	454	
192	fragment	455	
192	fragment	456	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
192	fragment	457	
192	fragment	458	
192	fragment	459	
192	fragment	460	
192	fragment	461	
192	fragment	462	
192	fragment	463	
193	fragment	464	
194	fragment	465	
195	fragment	466	
201	fragment	467	
201	fragment	468	
201	fragment	469	
201	fragment	470	
224	fragment	471	
251	fragment	472	
264	fragment	473	
264	fragment	474	
268	fragment	475	
268	fragment	476	
277	fragment	477	
282	fragment	478	
282	fragment	479	
282	fragment	480	
382	fragment	481	
405	fragment	482	
405	fragment	483	
405	fragment	484	
425	fragment	485	
425	fragment	486	
433	fragment	487	
460	fragment	488	
493	fragment	489	
500	fragment	490	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
558	fragment	491	
587	fragment	492	
587	fragment	493	
587	fragment	494	
587	fragment	495	
645	fragment	496	
645	fragment	497	
650	fragment	498	
685	fragment	499	
NT01SP0246 (45)	fragment	500	
NT01SP0246 (45)	fragment	501	
NT01SP0246 (45)	fragment	502	
NT01SP0246 (45)	fragment	503	
Spy0047	fragment	504	
SPy0080a	fragment	505	
spy0127	fragment	506	
Spy0272	fragment	507	
Spy0461	fragment	508	
Spy0611	fragment	509	
Spy0611	fragment	510	
Spy0611	fragment	511	
Spy0611	fragment	512	
Spy0611	fragment	513	
SPy0645	fragment	514	
Spy0686	fragment	515	
Spy0717	fragment	516	
Spy1073	fragment	517	
Spy1029	fragment	518	
SPy1260	fragment	519	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
Spy1613	fragment	520	
Spy1835	fragment	521	
Spy1835	fragment	522	
Spy2005	fragment	523	
Spy2005	fragment	524	
Spy2093	fragment	525	
Spy2093	fragment	526	
SPy2178	fragment	527	
24	fragment	528	
49	fragment	529	
57	fragment	530	
57	fragment	531	
64	fragment	532	
64	fragment	533	
64	fragment	534	
84	fragment	535	
98	fragment	536	
98	fragment	537	
98	fragment	538	
143	fragment	539	
143	fragment	540	
143	fragment	541	
149	fragment	542	
171	fragment	543	
188	fragment	544	
190	fragment	545	
191	fragment	546	
191	fragment	547	
191	fragment	548	
191	fragment	549	
191	fragment	550	
192	fragment	551	
192	fragment	552	
192	fragment	553	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
192	fragment	554	
198	fragment	555	
201	fragment	556	
201	fragment	557	
251	fragment	558	
251	fragment	559	
251	fragment	560	
262	fragment	561	
264	fragment	562	
282	fragment	563	
299	fragment	564	
362	fragment	565	
405	fragment	566	
405	fragment	567	
406	fragment	568	
545	fragment	569	
685	fragment	570	
spy0611	fragment	571	
spy0612	fragment	572	
spy0613	fragment	573	
spy0614	fragment	574	
spy0615	fragment	575	
spy0616	fragment	576	
spy0717	fragment	577	
spy0717	fragment	578	
spy0792	fragment	579	
spy1073	fragment	580	
spy1073	fragment	581	
NT01SP0908	fragment	582	
NT01SP0182	fragment	583	
NT04SP1422	fragment	584	
spy1111	fragment	585	
spy 0216	fragment	586	
spy1664	fragment	587	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
spy0861	fragment	588	
57 Chiron	fragment	589	
NT01SP0102	fragment	590	
35	Surface-exposed domain	591	
54	Surface-exposed domain	592	
70	Surface-exposed domain	593	
414	Surface-exposed domain	594	
421	Surface-exposed domain	595	
425	Surface-exposed domain	596	
426	Surface-exposed domain	597	
428	Surface-exposed domain	598	
433	Surface-exposed domain	599	
434	Surface-exposed domain	600	
437	Surface-exposed domain	601	
438	Surface-exposed domain	602	
439	Surface-exposed domain	603	
457	Surface-exposed domain	604	
461	Surface-exposed domain	605	
465-1	Surface-exposed domain	606	
465-2	Surface-exposed domain	607	
469	Surface-exposed domain	608	
472	Surface-exposed domain	609	
473	Surface-exposed domain	610	
474	Surface-exposed domain	611	
475	Surface-exposed domain	612	
477	Surface-exposed domain	613	
478	Surface-exposed domain	614	
486	Surface-exposed domain	615	
492	Surface-exposed domain	616	
494	Surface-exposed domain	617	
495	Surface-exposed domain	618	
535	Surface-exposed domain	619	
538	Surface-exposed domain	620	
540	Surface-exposed domain	621	

Table 1, cont.

GAS	type	SEQUENCE IDENTIFIER	
		amino acid	nucleotide
543	Surface-exposed domain	622	
553	Surface-exposed domain	623	
560	Surface-exposed domain	624	
561	Surface-exposed domain	625	
564	Surface-exposed domain	626	
565	Surface-exposed domain	627	
574	Surface-exposed domain	628	
576	Surface-exposed domain	629	
577-1	Surface-exposed domain	630	
577-2	Surface-exposed domain	631	
579	Surface-exposed domain	632	
586-1	Surface-exposed domain	633	
586-2	Surface-exposed domain	634	
587	Surface-exposed domain	635	
591	Surface-exposed domain	636	
592	Surface-exposed domain	637	
607	Surface-exposed domain	638	
609	Surface-exposed domain	639	
625	Surface-exposed domain	640	
626-1	Surface-exposed domain	641	
626-2	Surface-exposed domain	642	
636	Surface-exposed domain	643	
640	Surface-exposed domain	644	
643	Surface-exposed domain	645	
649	Surface-exposed domain	646	
653	Surface-exposed domain	647	
657	Surface-exposed domain	648	
663	Surface-exposed domain	649	
40N	full-length	930	
16p2	full-length	971	972
680	full-length	973	974
M30098		975	987
M3_0100		976	988
M3_0102		977	989
M3_0104		978	990
SPs0106		979	991
M6_0157		980	992

M6_0159		981	993
M6_0160		982	994
19224134		983	995
19224135		984	996
19224137		985	997
19224141		986	998

Table 2. GAS antigens present on the surface of multiple M types.

GAS antigen	M1(5)	M2(1)	M3(3)	M4(2)	M5(1)	M6(3)	M8(1)	M9(1)	M11(1)	M12(1)	M23(1)	ALL(20)
5	5	1	1	1	1	3		1		1		14
6	1	1	1			2				1	1	6
18	5			1	1		1					8
22	4	1	1	1	1	2				1		11
23	2		1	2	1	1				1		8
25	3				1		1		1	1		7
29						1				1	1	2
30	1			1	1	3				1		7
36	1				1					1		3
49	1				1	2						4
56	5	1		1	1	1	1	1				11
60	1					1					1	2
62	1	1		1		2				1		6
63				1		3						4
65			1	1		3						5
67	4	1		1	1	2		1			1	10
68	3	1				1	1		1	1		8
69	1	1	1	1	1	2				1		8
74						1						1
75	3	1		1	1	3						9
76	5	1	1		1	2		1		1		12
77	2	1	1	1	1	3	1			1		11
78			1			2						3
81	1	1	1		1	2						6
82						2						2

85					1	3						4
86										1		1
89	1				1	1				1		4
91					1					1		2
92		1			1					1		3
93	4	1			1	2				1		9
94	1	1		1		1	1			1		6
96	5	1		1	1	3			1	1		13
97	2		1	1	1	2						7
98	2					1				1		4
99	4	1	1	1	1	3	1		1	1		14
100	2				1	1						4
101	3	1				2						6
103	5	1		1	1	2	1	1		1	1	13
104	1				1	2						4
105	3	1			1		1	1				7
108	5	1			1	2	1	1		1		12
123	4	1		1		1				1		8
131	2									1		3
142	5	1		1	1	2			1	1	1	12
143	3	1		1	1	2						8
158	1					2						3
165						1				1		2
166	5	1	2	1	1	2	1			1	1	14
175	1			1		1						3
178	1	1				1						3
179				1		2				1		4
187	3	1			1		1		1			7
188	4	1		1	1	3	1	1	1			13
190	5	1		1		2		1	1	1	1	12

195	3	1			1	1				1		7
205		1				2				1		4
206	3		1	1	1	1				1		8
207	1											1
218		1				1				1		3
219		1			1							2
242	2	1			1	2				1		7
249	3	1		1	1	1				1		8
271						1						1
291	1	1				1				1	1	4
327						1				1		2
380	1											1
685	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t	n/t

n/t = not tested

Table 3. *In silico*-predicted surface-exposed proteins

GAS	SPY	no. aa	PSORT	TMD	Features	Annotation
5	spy0019	398	outside	0		putative secreted protein
6	spy0031	374	outside	0	RGD	putative choline binding protein
18	spy0130	215	membrane	2	LPXTG	hypothetical protein
22	spy0159	292	outside	0		hypothetical protein
23	spy0163	342	lipoprotein	0	lipoprotein	putative ABC transporter (lipoprotein)
25	spy0167	571	outside	0		streptolysin O precursor
29	spy0210	410	lipoprotein	0	lipoprotein	hypothetical protein (TGc, Transglutaminase/protease-like GBS682)
30	spy0212	234	outside	0		exotoxin G precursor
36	spy0252	439	lipoprotein	0	lipoprotein	putative sugar transporter sugar binding lipoprotein
49	spy0317	280	lipoprotein	0	lipoprotein	conserved hypothetical protein
56	spy0385	310	lipoprotein	0	lipoprotein	ferrichrome ABC transporter (ferrichrome-binding protein)
60	spy0436	232	membrane	1		putative exotoxin (superantigen)
62	spy0441	319	outside	0	RGD	conserved hypothetical protein (Predicted dehydrogenases)
63	spy0457	268	lipoprotein	0	lipoprotein	putative cyclophilin-type protein
65	spy0711	235	membrane	1		pyrogenic exotoxin C precursor, phage associated
67	spy0714	515	outside	0		putative adhesion protein
68	spy0737	2045	membrane	2	LPXTG	putative extracellular matrix binding protein

69	spy0778	270	lipoprotein	0	lipoprotein	putative ABC transporter (substrate-binding protein
74	spy1008	236	outside	0		streptococcal exotoxin H precursor
75	spy1032	805	outside	0		extracellular hyaluronate lyase
76	spy1037	318	outside	0		conserved hypothetical protein
77	spy1054	293	membrane	1	LPXTG-RGD	putative collagen-like protein (44%HUM)
78	spy1094	320	lipoprotein	0	lipoprotein	conserved hypothetical protein (S77609 probable adhesion)
81	spy1228	350	lipoprotein	0	lipoprotein	putative lipoprotein
82	spy1245	288	lipoprotein	0	lipoprotein	putative phosphate ABC transporter, periplasmic pho
85	spy1290	206	lipoprotein	0	lipoprotein	hypothetical protein
86	spy1294	415	lipoprotein	0	lipoprotein	putative maltose/maltodextrin-binding protein
87	spy1302	711	outside	0	RGD	putative cyclomaltodextrin glucanotransferase
88	spy1361	792	lipoprotein	0	lipoprotein	putative internalin A precursor
89	spy1390	351	lipoprotein	0	lipoprotein	putative protease maturation protein
91	spy1491	195	outside	0		conserved hypothetical protein
92	spy1558	207	lipoprotein	0	lipoprotein	hypothetical protein
93	spy1592	380	lipoprotein	0	lipoprotein	conserved hypothetical protein (maltose)
94	spy1633	535	outside	0		conserved hypothetical protein (hydrolase)
96	spy1795	294	lipoprotein	0	lipoprotein	putative ABC transporter (periplasmic binding protein)
97	spy1801	503	outside	0		immunogenic secreted protein precursor homolog
98	spy1882	284	lipoprotein	0	lipoprotein	putative acid phosphatase (secreted)

99	spy1979	440	outside	0		streptokinase A precursor
100	spy2000	542	lipoprotein	0	lipoprotein	surface lipoprotein
101	spy2007	306	lipoprotein	0	lipoprotein	putative laminin adhesion
103	spy2037	309	lipoprotein	0	lipoprotein	conserved hypothetical
104	spy2039	398	outside	0	RGD	pyrogenic exotoxin B (speB)
105	spy2066	498	outside	0		putative dipeptidase
108	spy0604	128	lipoprotein	0	lipoprotein	hypothetical protein
123	spy0510	308	membrane	2		putative sugar transferase
131	spy0601	282	membrane	1		putative endolysin, phage associated
142	spy0740	352	membrane	1		streptolysin S associated ORF
143	spy0747	910	membrane	2	LPXTG-RGD	conserved hypothetical protein
158	spy0843	1008	membrane	1	LPXTG	hypothetical protein (immunoreactive protein Se110)
165	spy1326	364	cytoplasm	0	RGD	conserved hypothetical protein
166	spy1357	217	membrane	1	LPXTG	protein GRAB (protein G-related alpha 2M-binding pr (delezioni)
171	spy1494	313	membrane	1	LPXTG	hypothetical protein
175	spy1577	357	membrane	1	LPXTG	3-dehydroquinate synthase
178	spy1697	240	membrane	1		hypothetical protein (extracellular serine protease)
179	spy1718	328	outside	0		putative esterase
187	spy1972	1165	membrane	2	LPXTG	putative pullulanase
188	spy1983	348	membrane	1		collagen-like surface protein (48%HUM)

190	spy2009	379	membrane	1	LPXTG	hypothetical protein
195	spy2043	271	membrane	1		mitogenic factor
206	spy0731	435	cytoplasm	0		putative enolase
207	spy0856	199	outside	0		putative peptidoglycan hydrolase
208	spy0857	235	outside	0		putative peptidoglycan hydrolase
210	spy0872	670	membrane	2	LPXTG	putative secreted 5'-nucleotidase
218	spy1006	444	membrane	1		putative lysin - phage associated
219	spy1007	225	cytoplasm	0		streptococcal exotoxin I
242	spy1306	419	membrane	1		maltose/maltodextrin-binding protein
249	spy1497	275	membrane	1		putative hemolysin
271	spy1850	316	membrane	1		putative esterase
291	spy1998	233	membrane	1		mitogenic exotoxin Z
327	spy0513	361	membrane	1		putative XAA-PRO dipeptidase; X-PRO dipeptidase
380	spy1813	995	outside	0		hypothetical protein (Glycosyl hydrolases family)
205	spy0453	310	lipoprotein	0	lipoprotein	metal binding protein of ABC transporter (lipoprotein)
685	spy0319	281	lipoprotein	0	lipoprotein	conserved hypothetical protein

LPXTG-RGD (SEQ ID NO:962)

LPXTG (SEQ ID NO:931)

Table 4A. Strain 2913 (M1)

2913 (M1)			
GAS	preimm	imm	D mean
190	194.42	474.68	280
188	189.71	459.15	269
5	191.08	437.36	246
18	143.38	383.45	240
gst98	206.91	437	230
gst123	164.85	366.01	201
76	162.58	360.52	198
105	162.91	350.52	188
103	137.1	311.95	175
urea104	194.85	362.78	168
urea131	197.29	363.6	166
166	144.84	307.9	163
77	210.6	370.99	160
49	141.14	297.46	156
23	144.17	298.99	155
187	204.06	356.99	153
100	139.63	290.75	151
166	150.22	297.12	147
142	143.75	289.89	146
96	171.49	316.8	145
96	196.77	336	139
108	146.23	278.17	132
99	144.54	276.02	131
67	152.03	279.31	127
gst93	134.44	260.24	126
96	139.88	265.67	126
99	214.7	337.29	123
5	142.95	265.27	122
56	147.28	268.99	122
22	172.13	285.71	114
99	131.02	242.52	112
25	258.24	367.75	110
5	234.57	343.37	109
gst143	210.59	318.47	108
117/40	173.8	270.35	97
195	156.71	252.98	96
142	162.6	256.58	94

195	144.64	235.8	91
40 native	131.04	220.34	89
206	208.21	295.19	87
158	342.78	427.07	84
313	213.24	294.56	81
97	237.96	318.85	81
30	228.04	305.4	77
69	224.85	301.84	77
117/40	192.49	263.31	71
242	132.82	202.24	69
91	216.61	284.68	68
29	138.08	204.89	67
urea210	354.84	417.89	63
81	196.88	256.93	60
249	137.91	197.54	60
75	201.32	259.63	58
101	136.92	194.98	58
219	202.88	260.49	58
gst175	201.69	258.37	57
gst68	217.7	272.94	55
85	201.23	256.11	55
gst6	187.31	241.82	55
178	120.12	172.44	52
89	220.52	271.56	51
82	204.97	253.43	48
63	200.53	247.1	47
165	304.53	346.53	42
36	183.7	225.55	42
23	211.65	252.18	41
179	132.17	171.24	39
86	262.12	294.72	33
gst62	205.71	237.43	32
291	153.15	181.2	28
5	259.32	285.62	26
65	182.47	208.38	26
327	234.5	257.44	23
205	216.71	237.39	21
gst94	198.09	218.63	21
218	145.43	165.25	20
urea207	330	348.95	19
88	252.44	269.1	17
gst60	187.92	197.82	10

urea271	345.63	351.41	6
380	152.43	158.11	6
92	246.3	250.22	4
gst78	190.44	179.39	-11
urea74	251.26	224.66	-27
208	315.54	107.25	-208

Table 4B.

3348 (M1)			
GAS	preimm	imm	D mean
56	154.54	565.12	411
gst98	173.97	508.69	335
96	132.72	449.76	317
76	151.87	426.15	274
190	159.41	415.54	256
18	140.84	387.87	247
gst123	162.67	398.89	236
5'	142.12	340.11	198
103	156.69	343.28	187
188	162.2	340.96	179
urea207	222.13	373.14	151
101	141.15	287.3	146
100	131.81	262.87	131
23	148.65	270.92	122
urea131	151.07	254.71	104
99	202.11	305.09	103
gst93	170.95	272.97	102
142	150.93	252.85	102
99	156.22	258.05	102
313	167.92	263.49	96
gst143	148.32	240.51	92
166	132.4	222.57	90
75	162.66	252.49	90
108	141.38	228.92	88
5	157.86	241.76	84
206	167.04	248.13	81
77	187.09	266.41	79
5	160.4	239.13	79
22	157.57	233.84	76
gst68	163.42	238.93	76

5	231.72	306.6	75
49	141.86	210.64	69
urea104	158.48	226.48	68
gst60	176.65	242.76	66
91	163.83	229.45	66
142	189.99	255.48	65
urea74	228.44	292.69	64
gst62	161.7	224.94	63
gst78	148.6	210.04	61
117/40	155.18	214.79	60
99	146.22	204.77	59
67	162.15	218.88	57
166	158.91	214.36	55
gst94	153.94	209.27	55
96	160.91	215.65	55
gst175	176.63	229.59	53
81	161.63	214.56	53
242	125.1	173.61	49
gst6	147.33	195.11	48
195	211.98	259.42	47
urea210	330.82	373.55	43
25	201.97	244.53	43
urea271	253.08	295.28	42
69	151.78	193.06	41
89	156.08	191.86	36
219	178.2	213.49	35
158	215.05	249.34	34
96	158.73	192.02	33
187	286.55	319.43	33
179	155.79	186.94	31
291	154.52	183.63	29
97	152.36	180.46	28
88	171.64	198.96	27
65	159.69	186.94	27
218	130.47	156.24	26
327	156.06	178.78	23
195	149.34	171.32	22
165	233.12	254.48	21
30	153.35	173.57	20
63	155.73	174.4	19
36	169.08	187.58	19
23	152.21	170.64	18

205	158.05	176.23	18
178	144.99	163.17	18
82	154.34	171.49	17
85	158.6	174.66	16
92	155.68	167.71	12
40 native	179.42	180.18	1
86	188.83	186.3	-3
29	185.03	178.03	-7
249	195.31	187.28	-8
117/40	189.5	181.12	-8
380	245.4	232.35	-13
105	266.98	225.36	-42
208	228.37	99.93	-128

Table 4C.

2726 (M2)			
GAS	preimm	imm	D mean
117/40	169.41	635.25	466
117/40	143.81	605.99	462
40/117	158.74	552.21	393
40 native	139.5	380.45	241
76	218.18	448.62	230
103	262.45	487.61	225
166	258.79	466.42	208
142	234.83	427.39	193
67	265.37	452.94	188
249	164.53	348.69	184
96	243.06	416.55	173
108	281.02	445.13	164
99	246.77	409.67	163
5	182.49	342.3	160
242	257.92	411.8	154
56	301.63	452.16	151
gst62	214.58	364.68	150
96	162.15	311.47	149
142	250.32	388.62	138
218	173.66	309.95	136
291	180	315.83	136
5	274.6	409.78	135
178	177.67	303.8	126

195	304.08	429.06	125
75	241.94	366.38	124
81	218.18	333.76	116
205	220.27	333.86	114
190	231.56	344.2	113
69	253.55	364.87	111
gst143	257.27	367.77	111
105	355.04	464	109
22	245.28	353.51	108
5	201.38	306.56	105
219	236.94	341.08	104
99	168.81	266.95	98
gst123	258.21	354.86	97
313	277.05	371.2	94
101	279.03	372.94	94
gst6	254.54	348.01	93
77	165.4	258.53	93
92	262.48	355.06	93
gst68	277.82	368.27	90
187	422.69	509.5	87
gst93	256.06	339.72	84
gst94	238.26	321.47	83
188	157.15	237.48	80
29	236.32	315.54	79
gst78	251.67	329.48	78
36	236.52	313.69	77
gst60	246.97	323.18	76
327	253.71	327.93	74
85	162.02	234.58	73
166	325.96	392.88	67
91	321	387.03	66
65	176.06	241.8	66
23	181.48	246.57	65
158	287.96	350.68	63
97	181.05	242.52	61
gst98	280.75	339.55	59
195	286.74	344.69	58
5	235.88	292.3	56
206	181.9	234.11	52
30	200.18	250.63	50
88	294.93	342.22	47
179	191.53	235.8	44

380	312.64	347.72	35
63	160.74	193.38	33
25	467.96	499.72	32
23	196.86	225.27	28
86	324.63	350.05	25
82	253.46	276.32	23
89	194.88	217.01	22
urea210	347.49	359.69	12
18	193	202.94	10
96	231.62	218.69	-13
99	188.69	174.54	-14
urea104	283.67	256.24	-27
urea271	339.03	309.14	-30
49	238.07	198.74	-39
urea74	295.64	239.71	-56
100	233.87	174.3	-60
urea207	349.08	286.99	-62
208	270.34	194.92	-75
165	284.04	189.33	-95
urea131	283.02	172.91	-110
gst175	358.69	140.1	-219

Table 4D.

3040 (M3)			
GAS	preimm	imm	D mean
gst78	165.03	282.6	118
206	194.34	305.19	111
5	194.31	300.67	106
99	202.73	307.71	105
77	184.54	288.6	104
81	167.66	264.24	97
97	186.2	281.97	96
gst6	170.39	261.83	91
65	181.08	269.99	89
5	185.58	272.75	87
69	175.92	261.83	86
22	178.99	261.25	82
23	184.44	266.54	82
76	177.15	257.16	80
188	190.11	267.91	78

gst62	170.45	244.75	74
5	210.52	279.67	69
190	173.27	242.19	69
86	198.28	263.6	65
313	185.15	250.41	65
96	176.77	236.77	60
219	196.43	254.4	58
327	188.42	241.55	53
gst68	190.6	242.94	52
gst93	183.17	232.96	50
85	167.91	217.39	49
89	186.18	234.75	49
gst175	194.08	241.57	47
30	192.04	239	47
88	196.06	240.78	45
gst60	180.47	222.06	42
75	167.31	204.26	37
91	203.53	237.3	34
82	189.88	222.78	33
gst94	191.97	221.13	29
205	202.78	229.36	27
36	168.15	194.4	26
63	245.76	269.86	24
92	184.31	189.26	5
gst143	187.36	158.17	-29

Table 4E.

3135 (M3)			
GAS	preimm	imm	D mean
166	110.13	394.61	284
166	107.56	382.36	275
urea131	114.39	174.55	60
103	106.67	166.24	60
158	122.44	175.29	53
5	122.45	163.77	41
23	121.36	161.69	40
195	107.19	147.11	40
urea104	111.36	147.25	36
97	122.67	156.78	34
25	137.45	171.09	34
77	124.69	155.7	31
99	130.22	159.98	30
56	106.91	132.45	26
108	106.5	131.78	25
206	124.87	147.61	23
188	123.84	145.6	22
gst78	119.2	140.45	21
29	108.28	128.56	20
63	126.8	147.01	20
gst98	110.66	129.86	19
313	126.91	145.44	19
96	111.43	129.23	18
5	128.79	145.75	17
49	108.24	124.96	17
99	105.45	122.12	17
65	124.99	141.46	16
22	123.41	139.65	16
gst62	123.52	139.61	16
67	105.52	121.18	16
249	106.75	122.12	15
291	110.37	125.59	15
142	108.05	123.01	15
5	111.21	125.9	15
219	120.29	134.55	14
urea210	133.06	146.91	14
96	120.11	133.93	14
81	119.08	132.81	14

99	106.41	120.04	14
urea271	121.47	134.91	13
5	126.45	139.79	13
gst143	124.56	137.5	13
gst93	120.4	132.87	12
76	122.05	134.36	12
86	127.78	140.06	12
195	111.37	122.97	12
218	109.75	121.32	12
gst6	121.82	133.37	12
117/40	107.5	118.49	11
23	106.15	116.48	10
30	125.53	135.78	10
urea74	114.41	124.63	10
gst123	110.37	120.25	10
18	107.52	117.28	10
85	121.62	131.21	10
100	107.13	116.71	10
89	123.02	132.21	9
117/40	109.32	118.42	9
69	122	130.75	9
142	107.56	116.23	9
179	108.33	116.82	8
75	120.38	128.56	8
327	123.25	130.86	8
40 native	107.54	114.46	7
101	109.87	116.68	7
190	122.5	129.25	7
178	106.51	112.97	6
urea207	115.66	122.06	6
380	107.39	112.46	5
88	128.77	133.4	5
165	118.63	122.64	4
gst94	122.63	126.36	4
91	127.34	130.97	4
gst60	122.62	125.76	3
205	124.21	127.27	3
92	122.58	125.45	3
82	126.38	128.68	2
36	119.01	119.08	0
gst68	126.75	124.32	-2
gst175	127.17	124.35	-3

242	107.62	103.99	-4
187	137.54	133.22	-4
105	129.67	120.74	-9
208	123.3	102.4	-21
96	200.67	137.45	-63

Table 4F.

2721 (M3)			
GAS	preimm	imm	D mean
166	99.66	226.57	127
166	97.13	198.93	102
103	98.07	140.45	42
23	125.99	159.23	33
25	103.57	129.87	26
195	98.17	124.06	26
gst62	112.5	138.24	26
206	117.76	134.66	17
urea74	119.74	136.43	17
100	99.01	114.15	15
urea104	98.42	112.99	15
22	121.44	135.44	14
urea131	100.88	114.48	14
5	114.52	127.44	13
108	98.18	109.68	12
gst6	111.2	122.5	11
86	115.72	125.96	10
85	116.36	126.5	10
76	113.43	123.38	10
82	113.19	122.65	9
77	111.92	121.12	9
81	112.89	121.83	9
99	116.07	124.65	9
5	117.05	125.62	9
40 native	98.41	106.2	8
29	95.88	102.59	7
89	119.9	126.61	7
97	117.88	124	6
158	101.16	107.22	6
75	114.86	120.8	6
142	96.49	102.22	6

219	114.33	120	6
36	112.86	118.36	6
195	96.7	102.04	5
99	100.79	106	5
380	96.79	101.99	5
5	97.09	102.14	5
gst78	113.72	118.69	5
63	114.63	119.19	5
gst94	115.6	120.06	4
96	100.89	105.27	4
142	97.66	101.88	4
40/117	100.57	104.78	4
30	116.53	120.66	4
291	96.92	100.84	4
96	120.86	124.77	4
99	97.66	101.3	4
67	97.27	100.85	4
242	98.01	101.18	3
205	115.96	119.07	3
165	103.2	106.27	3
49	99.45	102.5	3
18	99.52	102.47	3
190	95.57	98.34	3
179	98	100.56	3
101	101.58	104.06	2
327	113.06	115.17	2
117/40	99.08	101.18	2
69	118.09	120.08	2
249	99.02	100.83	2
96	108.49	110.08	2
218	97.68	99.27	2
178	96	97.27	1
gst123	116.42	117.54	1
5	118.39	119.44	1
gst93	116.77	117.66	1
56	106.85	107.74	1
187	106.45	107.29	1
92	120.72	120.96	0
23	105.99	105.83	-0
65	119.21	118.99	-0
urea271	117.8	117.44	-0
91	128.08	126.95	-1

urea210	117.69	115.93	-2
313	118.61	116.49	-2
gst175	116.58	114.1	-2
105	106.21	102.57	-4
urea207	120.6	116.8	-4
gst68	123.6	115.02	-9
88	128.51	117.28	-11
188	136.12	124.71	-11
gst98	124.32	112.82	-12
gst143	134.75	121.5	-13
gst60	135.21	119.59	-16

Table 4G.

2634 (M4)			
GAS	preimm	imm	D mean
5	152.3	397.43	245
142	200.73	391.1	190
77	172.99	361.68	189
97	145.78	326.33	181
179	138.12	312.92	175
40 native	206.13	375.63	170
67	189.05	357.68	169
96	208.98	376.29	167
103	193.23	359.56	166
99	152.4	312.85	160
65	149.8	309.86	160
30	214.78	369.95	155
5	149.11	304.03	155
23	182.23	331.41	149
gst94	206.2	342.91	137
22	197.28	330.34	133
gst175	209.41	335.86	126
75	195.62	315.74	120
5	139.66	254.37	115
99	179.89	294.17	114
188	200.24	313.88	114
166	172.06	285.61	114
63	270.77	381.55	111
gst62	207.91	318.39	110
56	193.55	303.44	110

166	184.73	289	104
18	206.67	306.03	99
117-40	210.77	306.45	96
249	120.31	215.54	95
gst123	244.06	334.45	90
gst143	187.74	277.76	90
99	200.38	289.4	89
190	206.02	292.65	87
206	354.53	440.07	86
101	189.01	266.42	77
gst6	222.38	299.01	77
gst60	232.53	309.02	76
195	187.95	263.95	76
76	201.83	277.53	76
81	171.97	245.71	74
96	192.32	262.29	70
gst93	227.24	295.46	68
gst68	225.46	292.55	67
49	256.66	323.34	67
gst78	198.87	264.72	66
69	195.79	260.58	65
108	233.34	298.06	65
195	180.52	241.19	61
219	167.02	226	59
105	308.53	365.92	57
291	117.07	172.87	56
142	186.09	239.15	53
5	170.81	223.48	53
25	346.28	397.53	51
23	195.69	245.88	50
85	316.65	359.95	43
91	249.58	292.39	43
165	268.89	309.85	41
29	129.86	168.67	39
gst98	230.53	269.14	39
380	220.91	257.34	36
187	344.99	378.74	34
158	382.35	415.03	33
218	123.8	154.83	31
urea104	255.47	281.99	27
36	207.01	233.44	26
242	258.94	284.93	26

92	284.03	309.39	25
205	250.69	275.3	25
96	243.12	265.11	22
327	184.43	205.62	21
88	257.24	277.7	20
100	245.7	263.38	18
313	248.95	264.11	15
urea210	541.55	550.33	9
82	232.34	233.15	1
86	275.19	260.17	-15
urea207	385.62	341.2	-44
urea74	298.46	248.91	-50
urea131	350.76	300.23	-51
208	385.47	324.29	-61
urea271	373.94	312.57	-61
89	327.1	115.03	-212

Table 4H.

2722 (M4)			
GAS	preimm	imm	D mean
23	97.88	248.22	150
23	137.39	278.58	141
69	140.97	221.83	81
188	141.87	216.09	74
97	155.1	226.67	72
77	144.43	196.68	52
108	107.91	159.37	51
96	98.02	145.04	47
117/40	109.57	153.16	44
101	111.9	155.44	44
96	129.15	170.61	41
99	97.92	138.62	41
96	100.25	134.01	34
5	143.26	176.88	34
65	147.92	180.96	33
142	108.6	140.41	32
242	105.99	136.91	31
99	136.6	166.22	30
166	109.37	138.29	29
40 native	103.23	130.36	27

25	125.33	151.38	26
18	107.22	131.87	25
5	108.33	131.9	24
56	112.92	135.88	23
30	146.84	169.5	23
85	142.96	165.36	22
291	119.24	141.22	22
166	107.78	129.32	22
gst175	130.2	150.6	20
75	135.54	155.88	20
195	108.7	128.47	20
5	136.9	156.55	20
218	111.31	130.3	19
380	115.11	133.89	19
100	110.41	128.88	18
76	132.33	150.76	18
89	155.47	171.89	16
249	119.23	134.44	15
142	105.42	120.62	15
urea104	126.08	140.6	15
92	150.99	164.91	14
206	129.28	142.78	14
103	111.19	124.28	13
219	141.75	154.3	13
urea74	141.75	154.3	13
195	112.18	123.63	11
49	124.86	134.86	10
29	114.18	122.71	9
178	107.62	115.69	8
67	116.67	124.72	8
190	110.36	117.59	7
22	137.2	144.24	7
187	129.17	135.91	7
36	136.41	141.8	5
gst143	158.83	161.79	3
urea131	137.35	140.19	3
63	154.01	156.15	2
gst123	154.11	154.7	1
gst68	128.03	127.53	-1
5	149.42	148.74	-1
81	155.94	153.99	-2
165	132.37	129.72	-3

gst94	161.13	157.93	-3
105	153.39	149.82	-4
gst78	153.06	148.37	-5
gst93	153.06	148.37	-5
40/117	113.26	108.52	-5
179	116.34	109.89	-6
205	149.42	141.91	-8
99	114.82	106.42	-8
gst98	128.83	120.31	-9
gst62	137.47	127.62	-10
158	147.38	137.25	-10
urea207	148.83	136.7	-12
gst60	155.88	143.3	-13
urea210	138.97	124.07	-15
82	189.74	173.22	-17
86	189.74	173.22	-17
327	150.01	132.88	-17
urea271	150.21	128.89	-21
313	155.89	122.48	-33
gst6	167.46	130.87	-37
88	219.73	153.36	-66
91	208.02	138.55	-69

Table 4I.

4883 (M5)			
GAS	preimm	imm	D mean
166	168.08	658.05	490
166	165.97	561.89	396
188	158.41	470.79	312
105	165.89	386.89	221
5	172.8	385.83	213
103	171.03	374.92	204
96	126.2	307.83	182
76	148.37	322.02	174
99	132.8	306.27	173
18	152.55	324.52	172
23	178.37	345.95	168
108	170.05	337.28	167
5	164.47	331.69	167
96	173.69	332.21	159

219	158.54	316.44	158
56	170.51	326.88	156
49	200.4	351.73	151
249	197.84	347.45	150
91	151.74	297.29	146
142	165.79	308.31	143
gst143	135.52	276.76	141
89	187.95	326.22	138
75	152.83	287.85	135
5	203.45	338.02	135
206	183.94	316.72	133
25	341.2	469.66	128
96	177.21	298.03	121
urea104	245.89	366.21	120
100	168.38	285.21	117
77	164	279.52	116
22	164.9	280.12	115
242	131.42	243.7	112
97	200.15	312.03	112
99	183.36	292.17	109
67	199.44	306.77	107
117/40	180.44	284.98	105
187	282.97	385.77	103
69	183.69	286.03	102
313	181.71	282.71	101
30	210.71	309.77	99
5	191.86	283.4	92
92	191.56	280.21	89
81	150.24	237.13	87
85	177.92	261.37	83
40 native	156.37	239.74	83
142	188.04	270.25	82
gst93	186.95	268.54	82
36	168.8	248.91	80
195	206.43	286.27	80
gst60	177.95	254.54	77
158	349.52	421.71	72
88	200.83	271.87	71
99	197.02	267.2	70
gst175	167.52	232.11	65
gst123	174.58	237.59	63
63	194.37	256.6	62

195	196.35	256.42	60
gst94	146.23	205.9	60
gst68	170.9	228.28	57
101	178.22	234.99	57
gst6	160.59	214.73	54
gst62	169.3	222.78	53
82	179.13	230.78	52
190	150.06	199.69	50
86	187.38	234.82	47
165	311.26	353.96	43
327	209.87	252.47	43
gst98	183.87	226.28	42
205	197.32	235.07	38
23	201.14	237.49	36
urea131	248.92	280.6	32
29	180.49	211.61	31
291	191.64	221.8	30
380	202.47	232.62	30
178	163.68	186.63	23
218	180.28	202.04	22
179	169.68	177.87	8
65	200.91	207.62	7
gst78	170.15	172.79	3
urea210	200.16	181.32	9
40/117	220.63	200.3	-20
urea271	261.57	236.15	-25
urea207	267.75	231.12	-37
urea74	267.05	190.64	-76

Table 4J.

5529 (M6)			
GAS	preimm	imm	D mean
gst143	216.28	565.3	349
5	222.8	565.32	343
gst93	218.01	491.78	274
96	209.54	474.43	265
190	209.35	441.87	233
gst78	194.51	426.44	232
81	188.65	386.95	198
5	207.04	402.67	196

gst6	208.73	401.26	193
77	250.02	438.29	188
99	201.93	389.12	187
gst175	243.91	429.67	186
205	203.33	387.87	185
5	213.11	395.56	182
313	240.06	420.21	180
65	227.98	398.01	170
75	242.58	408.08	166
gst62	268.21	433.22	165
97	218.49	383.14	165
85	203.95	363.71	160
30	224.9	383.85	159
gst60	250.6	407.31	157
188	231.95	380.57	149
76	252.76	400.5	148
63	273.29	410.45	137
gst94	335.69	464.28	129
22	239.55	365.06	126
327	221.26	344.22	123
69	239.73	361.33	122
gst68	240.06	359.89	120
89	258.01	371.03	113
82	221.43	328.02	107
23	267.29	356.28	89
206	269.61	355.03	85
92	261.57	311.12	50
36	238.33	281.48	43
219	313.65	329.71	16
88	301.67	297.26	-4
86	326.51	315.05	1
91	376.73	362.68	4

Table 4K.

2894 (M6)			
GAS	preimm	imm	D mean
96	142.82	468.85	326
166	197.13	497.83	301
5	161.1	442.77	282
5	182.49	421.83	239

178	133.19	366.71	234
142	166.61	397.1	230
gst143	175.94	401.93	226
99	205.67	425.2	220
gst98	175.67	394.34	219
190	140.21	350.66	210
urea271	288.29	498.29	210
76	150.29	353.93	204
96	136.19	336.53	200
85	142.74	339.31	197
166	162.51	358.59	196
gst6	156.35	351.42	195
195	134.89	328.14	193
gst123	194.87	383.44	189
142	200.89	389.07	188
5	142.96	330.07	187
77	188.83	365.44	177
67	184.89	354.35	169
urea104	157.41	322.31	165
30	167.55	325.49	158
81	157.48	311.3	154
165	234.51	386.33	152
108	131.77	277.94	146
96	136.85	282.62	146
242	145.15	290.03	145
179	126.79	270.66	144
65	141.1	281.88	141
49	137.46	271.59	134
158	226.68	357.1	130
75	198.96	327.74	129
205	166.31	290.27	124
188	140.51	264	123
gst93	180.92	303.53	123
22	165.74	285.77	120
103	135.97	255.97	120
5	136.8	256.57	120
gst78	166.6	280.92	114
101	145.42	256.97	112
97	161.53	272.37	111
99	142.55	252.24	110
249	154.21	260.33	106
99	155.11	259.89	105

63	183.32	288	105
urea74	273.9	376.33	102
218	193.12	289.73	97
82	151.09	247.6	97
291	230.77	326.18	95
56	133.13	226.56	93
313	164.4	257.09	93
gst62	213.44	301.49	88
29	146.63	232.54	86
195	146.49	229.21	83
69	169.17	251.36	82
117/40	116.48	191.3	75
40 native	153.81	222.75	69
100	140.04	206.26	66
89	190.08	255.3	65
23	276.7	340.78	64
urea207	235.8	298.53	63
117/40	308.59	370.9	62
327	162.83	219.35	57
206	207.34	262.2	55
gst175	184.7	231.44	47
18	196.97	242.89	46
gst68	185.03	226.78	42
urea210	319.86	361.09	41
380	153.11	189.62	37
91	253.81	283.56	30
urea131	388.76	401.45	13
92	228.84	228.61	-0
gst60	172.67	168.78	-4
23	201.52	189.19	-12
86	249.72	237.12	-13
88	232.21	218.65	-14
36	174.56	157.46	-17
gst94	357.68	320.75	-37
25	409.14	359.62	-50
219	279.52	199.04	-80
105	354.27	258.84	-95
208	249.85	99.36	50
187	518.52	256.39	-262

Table 4L.

3650 (M6)			
GAS	preimm	imm	D mean
5	146.42	493.86	347
96	194.48	461.78	267
5	170.83	436.96	266
5	193.67	459.02	265
166	198.73	374.92	176
96	145.29	311.33	166
108	145.24	307.56	162
5	174.55	336.6	162
103	133.82	295.63	162
166	159.73	309.82	150
67	187.77	335.44	148
96	143.54	282.31	139
99	156.87	285.37	129
165	242.31	368.83	127
158	230.31	355.33	125
77	169.06	293.47	124
142	184.4	308.65	124
99	186.35	309.24	123
242	150.89	260.42	110
179	146.46	255.93	109
85	184.74	290.71	106
65	176.52	277.49	101
63	190.49	290.04	100
101	146.2	243.55	97
urea104	183.51	278.91	95
75	205.16	293.63	88
49	143.02	228.44	85
100	156.63	240.89	84
30	177.71	259.73	82
188	162.5	243.73	81
99	216.69	293.47	77
327	166.96	242.38	75
190	113.74	188.14	74
81	167.4	241.78	74
gst62	199.1	273.1	74
56	152.05	225.58	74
gst78	190.66	260.53	70
206	202.02	270.02	68
195	160.32	225.37	65
18	201.92	265.78	64

142	180.11	242.07	62
380	152.3	213.67	61
gst143	169.13	227.88	59
195	144.93	203.08	58
69	177.22	234.26	57
205	162.95	216.28	53
82	170.26	219.96	50
gst93	184.97	234.59	50
249	158.28	204.93	47
76	197.51	243.79	46
40 native	164.5	210.53	46
gst6	182.47	227.05	45
178	148.92	193.29	44
urea271	239.45	283.82	44
97	192.1	230.07	38
23	213.62	243.44	30
22	190.7	219.32	29
92	205.9	234.18	28
291	242.82	267.35	25
313	169.51	193.55	24
218	184.47	204.73	20
23	280.69	300.66	20
86	217.84	233.64	16
gst175	175.99	190.91	15
gst60	178.72	193.42	15
89	209.93	224.22	14
urea74	214.59	228.03	13
36	188.1	198.97	11
219	210.94	211.99	1
29	134.98	133.36	-2
91	238.68	235.25	-3
88	212.52	206.02	-7
urea210	217.74	206.08	2
gst123	186.58	173.22	3
urea131	365.24	345.44	-20
gst68	188.41	162.3	-26
25	383.59	355.37	-28
gst98	200.15	164.33	-36
gst94	281.97	228.52	-53
urea207	236	174.46	-62
117/40	294.54	227.06	-67
105	322.94	230.21	-93

40/117	401.06	230.13	71
187	497.21	260.15	-237

Table 4M.

2725 (M8)			
GAS	preimm	imm	D mean
117/40	119.7	361.05	241
117/40	129.54	366.6	237
40 native	133.34	346.36	213
103	143.97	350.55	207
105	154.68	342.64	188
188	118.31	281.98	164
56	158.29	314.14	156
187	181.5	316.19	135
25	240.65	370.84	130
108	138.43	260.16	122
40/117	142.79	259.15	116
99	163.46	266.11	103
77	130.54	229.17	99
gst94	156.84	253.22	96
gst68	206.05	293.62	88
18	139.11	226.15	87
166	132.95	213.94	81
urea131	200.54	279.46	79
urea104	208.37	286.62	78
67	156.01	228.5	72
gst123	188.09	259.19	71
96	156.69	227.47	71
249	150.34	215.57	65
5	134.04	195.64	62
gst143	113.16	170.42	57
76	108.48	162.12	54
gst93	111.11	164.58	53
gst62	195.2	246.49	51
96	118.26	167.04	49
99	130.92	175.71	45
242	140	183.54	44
158	322.87	361.21	38
166	141.26	179.03	38
313	111.76	148.58	37

190	112	147.89	36
23	161.53	197.22	36
195	136.65	170.16	34
101	135.83	166.46	31
291	147.4	177.09	30
218	144.77	172.56	28
gst98	204.99	231.92	27
219	110.3	136.17	26
142	149.82	175.07	25
22	111.55	136.38	25
5	135.66	158.73	23
92	118.72	140.78	22
75	113.07	135.09	22
29	132.66	154.11	21
69	116.56	137.57	21
178	117.93	137.5	20
49	154.54	173.45	19
gst6	108.85	126.92	18
36	107.48	125.36	18
100	160.26	177.87	18
91	112.25	129.1	17
5	140.39	156.81	16
81	109.92	125.21	15
380	143.37	157.01	14
gst78	109.56	121.56	12
gst60	110.33	119.92	10
179	142.13	150.44	8
96	212.43	219.4	7
205	111.13	117.85	7
30	137.88	143.78	6
5	156.13	161.6	5
82	115.91	121.29	5
206	141.1	145.37	4
327	116.21	120.45	4
85	124.15	128.17	4
99	121.93	125.38	3
23	132.52	134.38	2
65	123.06	124.46	1
86	123.24	124.2	1
97	148.35	148.02	-0
63	130.88	128.42	-2
88	128.87	125.08	-4

89	142.83	137.31	-6
195	145.99	134.36	2
urea210	341.21	320.62	-21
142	142.04	115.27	-27
urea74	240.92	200.02	-41
urea271	334.19	252.53	-82
165	301.17	218.36	-83
urea207	348.18	259.49	-89
gst175	240	110.44	30
208	335.19	185.85	49

Table 4N.

2720 (M9)			
GAS	preimm	imm	D mean
117/40	141.02	457.48	316
117/40	145.73	459.86	314
40/117	165.72	421.99	256
188	148.05	393.4	245
103	193.76	316.13	122
40 native	139.6	257.15	118
67	195.71	309.91	114
190	148.58	258.46	110
56	195.41	302.64	107
105	199.31	299.11	100
76	144.96	239.8	95
5	140.99	234.64	94
108	194.22	278.09	84
gst6	152.71	231.66	79
219	154.98	228.78	74
gst62	155.74	227.55	72
249	179.3	249.93	71
gst143	156.61	223.44	67
gst94	159.33	222.14	63
96	134.8	196.78	62
96	161.74	219.43	58
142	183.78	239.61	56
22	147.85	203.36	56
166	183.15	238.61	55
18	161.74	216.84	55
69	155.21	209.87	55

36	140.97	195.19	54
166	180.38	234.07	54
81	144.72	197.76	53
gst93	157.96	210.45	52
327	159.07	211.23	52
242	180.72	231.33	51
5	142.32	192.3	50
77	158.26	206.73	48
75	149.1	195.09	46
gst60	158.26	202.34	44
313	164.87	207.49	43
29	165.78	208.37	43
178	153.97	196.08	42
85	150.83	192.41	42
23	159.35	200.37	41
5	194.91	232.58	38
218	170.82	208.14	37
142	190.44	226.92	36
101	195.32	231.76	36
195	200.27	236.02	36
5	161.19	196.51	35
gst68	193.71	228.96	35
gst123	200.92	235.71	35
205	164.46	199.19	35
97	169.12	202.91	34
99	133.4	165.99	33
63	158.28	189.81	32
99	200.88	231.43	31
91	175.71	204.73	29
291	165.4	193.78	28
99	145.56	173.44	28
206	148.07	172.25	24
92	188.4	207.68	19
25	323.41	342.29	19
gst78	159.86	178.6	19
100	159.03	176.45	17
82	171.61	186.09	14
89	159.72	174.07	14
30	169.35	182.78	13
gst98	199.05	211.94	13
187	276.98	289.86	13
65	145.41	156.44	11

23	147.37	158.25	11
49	180.46	190.17	10
urea104	215.48	225	10
179	171.29	180.75	9
195	195.69	204.26	9
urea131	195.32	202.88	8
158	254.04	252.18	-2
86	203.55	198.07	-5
380	227.51	221.75	-6
urea210	254.72	245.63	-9
88	202.8	187.02	6
165	252.45	224.98	-27
urea74	216.05	170.45	-46
urea271	271.47	218.87	-53
96	258.44	203.18	-55
urea207	273.74	213.67	-60
208	271.52	179.66	-92
gst175	230.39	119.02	11

Table 4O.

2727 (M11)			
GAS	preimm	imm	D mean
187	140.08	317.29	177
188	118.52	285.5	167
190	120.67	272.78	152
gst68	110.12	221.3	111
142	110.78	215.59	105
25	128.41	231.82	103
99	108.65	206.18	98
96	113.81	207.75	94
40 native	130.45	219.92	89
103	109.6	187.48	78
291	106.95	184.41	77
208	137.32	213.53	76
gst143	117.64	191.02	73
5	109	178.51	70
195	115.93	181.44	66
179	109.29	171.37	62
91	116.4	175.37	59

gst60	113.66	171.71	58
206	126.96	183.01	56
117-40	114.35	170.1	56
77	126.88	179.9	53
56	110.53	162.41	52
99	121.25	172.91	52
142	108.23	159.4	51
5	125.9	175.69	50
88	124.25	171.66	47
166	109.18	155.79	47
85	127.34	171.98	45
218	107.96	150.44	42
327	117.91	159.29	41
23	112.83	153.62	41
313	117.46	156.32	39
76	115.83	154.67	39
105	136.05	171.93	36
gst93	119.6	153.62	34
96	124.82	158.27	33
380	109.15	141.7	33
urea271	127.42	159.06	32
69	122.08	152.38	30
249	108.85	138.01	29
65	128.86	157.38	29
36	112.53	141	28
29	114.25	142.13	28
97	125.05	152.44	27
67	110.84	138.14	27
gst175	108.81	135.59	27
92	120.63	146.68	26
158	114.09	139.35	25
22	118.34	142.91	25
166	110.16	133.3	23
86	121.27	144.27	23
89	130.95	152.72	22
5	134.47	156	22
gst78	123.05	144.55	22
gst6	115.84	136.22	20
gst94	110.3	129.83	20
108	110.33	128.88	19
81	116.42	134.69	18
242	109.07	125.55	16

63	125.89	142.04	16
30	121.28	136.18	15
urea131	114.53	129.05	15
5	134.59	148.71	14
75	116.08	130.14	14
gst123	108.56	121.85	13
urea104	113.37	126.08	13
195	108.57	119.53	11
219	117.54	127.94	10
100	106.44	115.69	9
82	120.43	129.57	9
49	107.09	115.98	9
205	121.35	130.04	9
18	107.52	114.29	7
101	109.29	116.03	7
gst62	116.1	122.71	7
99	109.33	112.71	3
urea207	114.74	117.31	3
gst98	115.38	117.54	2
96	114.2	115.91	2
23	120.21	121.22	1
165	113.41	113.93	1
urea74	122.75	122.87	0
urea210	148.43	145.97	-2

Table 4P.

2728 (M12)			
GAS	preimm	imm	D mean
gst94	251.84	680.42	429
142	338.89	734.89	396
195	336.24	657.87	322
206	183.39	503.53	320
91	215.56	526.39	311
117/40	301.43	593.03	292
86	237.22	494.71	257
179	348.77	604.7	256
gst93	206.33	459.47	253
108	285.59	522.09	237
5	394.13	617.66	224
249	346.58	563.49	217

gst123	276.94	486.97	210
92	177.33	379.12	202
327	182.42	383.27	201
195	387.46	582.58	195
166	320.52	513.12	193
40/117	378.99	568.74	190
5	142.19	330.38	188
205	188.72	376.65	188
103	260.61	443.88	183
gst68	276.36	458.41	182
29	443.15	619.08	176
89	214.67	386.61	172
190	159.65	325.96	166
165	330.05	492.13	162
urea131	242.23	394.87	153
99	242.7	389.56	147
291	317.66	462.75	145
25	403.44	545.75	142
22	197.09	327.2	130
36	174.89	304.42	130
142	396.87	515.19	118
gst62	263.34	381.18	118
242	314.13	424.94	111
69	211.17	321.93	111
166	411.87	518.49	107
77	162.29	264.68	102
gst98	236.21	333.27	97
218	404.99	497.96	93
76	159.37	251.91	93
23	238.76	325.36	87
30	181.8	266.09	84
96	382.18	465.7	84
gst6	189.43	269.12	80
40 native	389.93	469	79
380	272.74	351.3	79
88	223.66	297.18	74
219	171.87	242.53	71
gst78	204.47	268.61	64
117/40	323.77	386.97	63
96	244.24	305.7	61
81	195.87	256.82	61
18	202.14	259.92	58

urea207	328.03	385.04	57
5	153.34	206.87	54
158	275.34	326.03	51
67	420.95	468.25	47
97	174.29	220.69	46
82	212.48	255.68	43
urea74	316.31	357.5	41
75	189.81	227.98	38
100	217.94	253.84	36
gst143	196.9	232.63	36
188	178.03	208.6	31
63	176.05	206.46	30
313	178.37	201.14	23
208	348.19	368.12	20
49	228.91	245.51	17
101	348.39	363.93	16
5	175.01	183.36	8
65	172.37	178.02	6
99	370.31	375.14	5
85	170.05	174.72	5
urea210	400.11	404.21	4
23	154.14	155.97	2
99	158.02	156.91	
96	155.2	150.51	-5
187	440.44	430.29	0
gst60	217.51	203.6	4
urea271	444.89	373.82	-71
105	473.03	380.39	-93
56	284.4	180.93	03
178	518.6	407.23	11
urea104	463.47	337.94	26
gst175	401.85	125.16	-277

Table 4Q.

DSM 2071 (M23)			
GAS	preimm	imm	D mean
67	128.35	359.3	231
166	124.38	326.64	202
190	125.36	315.81	190
gst60	121.15	276.58	155

103	114.95	231.03	116
142	118.53	234.37	116
29	116.02	223.84	108
291	121.62	215.87	94
gst6	125.51	211.9	86
313	122.76	202.26	80
56	127.35	201.01	74
380	115.41	180.08	65
97	137.33	200.6	63
96	169.18	229.08	60
99	125.69	184.5	59
gst78	129.66	188.11	58
23	119.91	177.68	58
188	132.06	188.54	56
gst68	118.31	174.37	56
206	162.71	216.36	54
91	127.52	179.67	52
urea104	128.64	180.29	52
89	150.16	200.54	50
gst175	120.66	171.02	50
81	134.57	182.95	48
88	125.63	173.6	48
166	123.8	171.21	47
gst93	130.31	176.42	46
117-40	132.35	177.38	45
99	153.44	198.42	45
195	137	179.72	43
96	138.79	180.84	42
242	137.11	177.52	40
92	127.34	165.99	39
22	128.37	166.75	38
195	117.66	154.18	37
40 native	192.07	228.3	36
205	130.34	166.16	36
23	149.18	183.91	35
gst62	126.42	160.49	34
165	136.68	170.31	34
gst143	131.51	164.59	33
86	126.07	158.41	32
101	113.82	145.37	32
5	153.64	184.82	31
99	127.62	158.78	31

77	149.48	179.87	30
82	124.97	154.96	30
18	124.84	154.58	30
69	117.82	146.47	29
158	151.39	178.9	28
urea271	141.04	168.16	27
5	117.67	144.56	27
249	131.83	158.42	27
85	160.45	186.93	26
gst94	122.06	148.04	26
urea131	147.64	172.92	25
5	143.5	168.5	25
25	130	154.87	25
76	121.55	144.69	23
100	118.11	140.17	22
65	148.35	170.11	22
gst98	125.6	147.13	22
urea74	140.84	161.11	20
5	184.82	205.08	20
96	125.15	144.29	19
75	127.09	145.9	19
219	127.33	144.11	17
30	149.61	165.06	15
327	127.95	142.74	15
49	124.67	136.44	12
179	121.78	133.02	11
63	163.28	173.73	10
gst123	139.21	149.4	10
36	127.54	136.23	9
urea207	139.53	145	5
218	127.08	126.81	-0
urea210	202.86	194.93	-8
105	192.62	181.45	1
208	163.58	145.96	8
142	179.95	136.43	-44
187	217.14	165.85	-51
108	336.37	249.51	-87

Table 4R.

HYPOCAPSULATED (M23)

GAS	preimm	imm	D mean
166	147.07	320.25	173
166	115.19	287.83	173
5	165.76	307.64	142
89	174.86	292.62	118
96	168.28	280.46	112
103	110.55	216.99	106
158	139.81	230.42	91
40 native	147.21	223.64	76
gst60	127.79	202.72	75
23	160.79	233.16	72
97	168.83	239.57	71
77	153.6	224.28	71
67	113.23	176.15	63
5	174.46	233.28	59
99	174.53	226.03	52
30	171.57	219.07	48
5	178.58	224.21	46
108	108.5	152.72	44
gst68	114.55	153.63	39
249	127.45	166.15	39
117-40	115.85	153.66	38
291	119.47	156.88	37
22	117.77	149.66	32
65	158.28	189.33	31
gst143	118.08	148.26	30
56	113.9	144	30
gst175	113.63	142.1	28
75	113.43	141.09	28
63	164.81	191.66	27
188	112.28	138.93	27
81	108.59	135.07	26
urea104	115.9	142.18	26
gst94	110.32	135.77	25
380	117.06	140.96	24
gst93	116.03	139	23
142	123.42	145.26	22
urea74	126.25	147.41	21
76	114.73	133.92	19
25	177.41	196.44	19
105	135.7	153.54	18
gst6	111.86	129.37	18

85	169.93	186.43	17
gst62	122.14	137.32	15
99	114.17	127.97	14
18	106.85	120.35	14
190	128.79	140.94	12
242	111.36	122.31	11
195	109.23	119.63	10
69	118.95	128.27	9
218	114.25	123.24	9
205	131.65	140.25	9
36	113.81	121.64	8
gst123	115.49	122.73	7
92	131.82	139.05	7
29	115.59	122.77	7
101	114.03	118.95	5
gst98	109.62	114.26	5
23	103.38	107.36	4
165	153.83	157.05	3
99	103.32	105.76	2
96	116.94	119.33	2
5	108.81	110.82	2
88	135.87	136.8	1
206	155.86	155.67	-0
urea131	140.85	140.3	
313	128.87	127.82	
82	136.42	133.65	-3
219	113.18	110.38	-3
gst78	120.15	117.09	-3
142	107.3	103.71	-4
49	108.29	104.42	-4
179	122.91	115.04	-8
195	114.49	105.38	-9
91	133.5	123.34	0
86	158.27	147.33	1
96	118.41	106.12	2
100	114.44	100.4	4
327	128.8	110.11	9
187	190.7	170.46	-20
urea271	154.79	128.67	-26
urea207	170.88	122.57	-48
urea210	299.43	236.67	-63
208	247.37	167.41	-80

Table 5. Percent identity of GAS40 proteins compared to reference strain SF370 (M1)

Strain	nucleotide differences	amino acid differences	M type	% identity
3280	0	0	1	100
3789	0	0	78	100
3348	0	0	1	100
2913	0	0	1	100
2580	0	0	1	100
2719	0	0	1	100
4959	9	3	77	100
2722	5	2	4	100
2894	10	4	6	100
3776	8	4	44	99.6
3650	11	4	6	99.6
5529	10	4	6	99.6
2728	9	5	4	99.5
2725	9	5	8	99.5
2720	9	4	9	99.5
2724	7	4	6	99.5
DSM2071	12	5	23	99.4
2634	12	6	1	99.4
4436	10	5	28	99.4
4883	13	5	5	99.4
5481	13	5	44	99.4
5476	10	4	89	99.4
4538	21	6	50	99.3
5455	21	6	62	99.3
2721	23	8	3	99.3
3040	23	7	3	99.2
5531	17	8	75	99.2
4088	15	7	ND	99.2
3135	22	8	3	99.2
2727	19	9	11	99.0
2726	101	25	2	95.9

Table 6A.

Group	Survival rate 7 days post-infection												
	1	2	3	4	5	6	7	Fatalities	total mice	% survival	strain	cfu/topo	
1	0	7	2	0	0	0	0	9	10	10	2071	30	HIS stop
2	0	1	2	0	0	0	0	0	9	66	2071	30	M23
3	0	4	1	0	0	0	0	5	10	50	2071	30	40N (SEQ ID NO:930)

Table 6B.

Group	Survival rate 7 days post-infection												
	1	2	3	4	5	6	7	Fatalities	total mice	% survival	strain	cfu/topo	
1	0	0	7	0	0	0	0	7	10	30	2071	30	HIS stop
2	0	0	0	0	0	0	0	0	10	100	2071	30	M23
3	0	0	2	0	0	0	0	2	10	80	2071	30	40N (SEQ ID NO:930)

Table 7. GAS proteins identified after protease digestion of the bacterial cell surface (LPXTG, SEQ ID NO:931; RGD LPXTG, SEQ ID NO:932)

GAS	SPY	M1=1697	PSORT prediction	TMD	Features	Description	FACS response (SF370)	Free trypsin	Free prot. K	Immob. prot. K	Immob. trypsin
4	spy0015	gi-15674261	membrane	1		putative cell division protein	Negative	x			
5	spy0019	gi-15674263	outside	0		putative secreted protein	Positive				x
15	NA	gi-23503478		2	LPXTG	collagen binding protein	Positive		x		
16	spy0128	gi-15674343	membrane	1		hypothetical protein	Positive		x		
23	spy0163	gi-15674368			lipoprotein	putative ABC transporter (lipoprotein)	ND	x			
24	spy0165	gi-15674370	secreted	1		nicotine adenine dinucleotide glycohydrolase precursor	Positive		x	x	
25	spy0167	gi-15674372			outside	streptolysin O precursor	ND	x			x
40	spy0269	gi-15674449	membrane	2		putative surface exclusion protein	Positive	x			
49	spy0317	gi-15674482	lipoprotein	0	lipoprotein	conserved hypothetical protein	Positive		x		
54	spy0351	gi-15674505	lipoprotein	3	lipoprotein	hypothetical protein	ND	x			
57	spy0416	gi-15674549	cell wall	1	LPXTG	putative cell envelope proteinase	Positive	x	x		
63	spy0457	gi-15674576	lipoprotein	0	lipoprotein	putative cyclophilin-type protein	Positive				x

64	gi-15674586	secreted	0		putative 42 kDa protein	Positive	x	x		
68	gi-15674788	membrane	2	LPXTG	putative extracellular matrix binding protein	Positive	x			
72	gi-15674925	membrane	1		putative ABC transporter (binding protein)	Positive	x			
84	gi-15675229	lipoprotei n	0	lipoprotein	putative amino acid ABC transporter, periplasmic am	Positive	x		x	
86	gi-15675247	lipoprotei n	0	lipoprotein	putative maltose/maltodextrin -binding protein	Positive	x			
87	gi-15675254		0	outside - RGD	putative cyclomaltodextrin glucanotransferase	ND				x
89	gi-15675314	lipoprotei n	0	lipoprotein	putative protease maturation protein	Positive	x			
98	gi-15675700	lipoprotei n	0	lipoprotein	putative acid phosphatase	Positive	x	x		
102	gi-15675798			outside	inhibitor of complement-mediated lysis	ND				x
103	gi-15675810	lipoprotei n	0	lipoprotein	conserved hypothetical	Positive	x			
108	gi-15674686	lipoprotei n	0	lipoprotein	hypothetical protein	Positive				x
143	gi-15674798	cell wall	2	RGDLPXTG	conserved hypothetical protein	Positive	x	x	x	
149	gi-15674825	membrane	1		putative large conductance mechanosensitive channel	Positive	x			

152	spy0802	gi-15674844	membrane	1		hypothetical protein	Negative		x		
157	spy0836	gi-15674871	membrane	1		conserved hypothetical protein	Positive			x	
158	spy0843	gi-15674877	cell wall	1		hypothetical protein	Positive	x			
163	spy1154	gi-15675130	membrane	2		hypothetical protein	Positive	x	x		x
166	spy1357	gi-15675290	cell wall	1	LPXTG	protein GRAB (protein G-related alpha 2M-binding protein)	Positive	x			
168	spy1370	gi-15675302	membrane	1		putative deacetylase	ND				x
171	spy1494	gi-15675398	cell wall	1	LPXTG	hypothetical protein	ND	x	x		
177	spy1649	gi-15675521	membrane	1		putative penicillin-binding protein 1a	Positive				x
188	spy1983	gi-15675773	cell wall	1	LPXTG	collagen-like surface protei	Positive	x	x		
190	spy2009	gi-15675795	cell wall	1	LPXTG	hypothetical protein	Positive	x	x		
191	spy2010	gi-15675796	cell wall	1	RGDLPXTG	C5A peptidase precursor	Positive	x	x		
192	spy2018	gi-15675799	cell wall	2	LPXTG	M protein type 1	Positive	x	x	x	
193	spy2025	gi-15675802	membrane	1		immunogenic secreted protein precursor	Negative	x			
194	spy2032	gi-15675807		1	membrane	putative ATP-binding cassette transporter-like protein	ND	x			
195	spy2043	gi-15675815		1	membrane	mitogenic factor	ND				x
198	spy2184	gi-15675919	membrane	2		conserved hypothetical protein	Negative	x			
201	spy2216	gi-15675945	membrane	1		putative serine protease	Positive	x			

224	spy1044	gi-15675040	membrane	2		hypothetical protein	ND			x	
251	spy1520	gi-15675420	membrane	1		putative cell division protein	Positive	x	x		
259	spy1586	gi-15675473	membrane	1		putative beta-galactosidase	ND	x			
262	spy1643	gi-15675516	membrane	1		hypothetical protein	ND	x			
264	spy1686	gi-15675546	membrane	1		hypothetical protein	ND	x			
268	spy1798	gi-15675635		1	membrane	hypothetical protein sharing similarity with severa	ND	x			
277	spy1939	gi-15675742	membrane	1		hypothetical protein	Positive	x			
282	spy2033	gi-15675808	membrane	1		hypothetical protein	Positive	x	x		
299	spy1188	gi-15675157	membrane	1		putative citrate lyase, beta subunit	ND	x			
382	spy1842	gi-15675668		1	membrane	putative signal peptidase I	ND	x			
405	spy1028	gi-15675026	membrane	1		putative acetoin dehydrogenase (TPP-dependent) beta	Positive	x			
406	spy1031	gi-15675028	membrane	1		putative dihydrolipoamide dehydrogenase, component	Positive	x			
425	spy0184	gi-15674389	membrane	6		putative glycine-betaine binding permease protein	Positive				x
433	spy0277	gi-15674455	cell wall	3		putative glutamine-binding periplasmic protein	Positive	x			
460	spy0572	gi-15674662	membrane	7		beta-glucoside permease IIABC component	ND		x		
469	spy0645	gi-15674715	membrane	4		putative cell-division protein	ND	x			

493	spy0743	gi-15674794	membrane	6		hypothetical protein	ND			x	
500	spy1410	gi-15675330	membrane	2		putative 1-acylglycerol-3-phosphate O-acyltransferase	ND	x			
545	spy1740	gi-15675589	membrane	3		putative mannose-specific phosphotransferase system	ND	x			
558	spy1109	gi-15675091	membrane	10		putative L-malate permease	ND			x	
587	spy1315	gi-15675263	membrane	4		hypothetical protein	ND	x			
645	spy2029	gi-15675805	membrane	4		putative ABC transporter (ATP-binding protein)	ND	x	x		
650	spy2120	gi-15675870		11	membrane	putative integral membrane protein	ND				
685	spy0319	gi-15674483	lipoprotein	0		conserved hypothetical protein	Positive	x			
362-1	spy1461	gi-15675369	secreted	0		hypothetical protein	Positive	x			
NS	spy0080a	gi-15675947				ribosomal protein L17	ND				
NS	spy0272	gi-15674451				30S ribosomal protein S7	ND				
NS	spy0461	gi-15674580				50S ribosomal protein L1	ND				
NS	spy0611	gi-15674691	cytoplasm			putative translation elongation factor EF-Tu	ND	x	x		
NS	spy0717	gi-15674775	cytoplasm			50S ribosomal protein L31	ND	x			
NS	spy0792	gi-15674835	cytoplasm			conserved hypothetical protein - possibly involved in cell wall localization and side chain	ND			x	

						formation					
NS	spy1029	gi-15675027				putative dihydrolipoamide S-acetyltransferase	ND				
NS	spy1073	gi-15675065	cytoplasm			50S ribosomal protein L7/L12	ND	x			
NS	spy1260	gi-15675219				hypothetical protein	ND				
NS	spy1613	gi-15675492				conserved protein - function unknown	ND				
NS	spy1835	gi-15675662				putative thioredoxin	ND				
NS	spy2005	gi-15675792				hypothetical protein	ND				
NS	spy2093	gi-15675850				putative elongation factor TS	ND				
NS	spy2178	gi-15675914				30S ribosomal protein S4	ND				
45	NT01SP 0246	gi-1420859, gi-19745421, gi-28895133, gi-56808335				oligopeptide permease (lipoprotein)		x			x
	spy0047	gi-15674286				putative signal peptidase I (lepA)		x			
NS	spy0127	gi-5674342						x			
NS	spy0686	gi-15674750									x

Table 8. GAS proteins identified after antibiotic treatment and overproduction of membrane vesicles

GAS	spY	M1=1697	PSORT prediction	TMD	Features	Description	FACS response (SF370)	Control	Penicillin	Vancomycin
5	spy0019	gi-15674263	outside	0		putative secreted protein	positive	x	x	
10	spy0097	gi-15674320	membrane	1		putative penicillin-binding protein 1b	positive		x	
23	spy0163	gi-15674368	lipoprotein	0	lipoprotein	putative ABC transporter (lipoprotein)	negative	x	x	x
24	spy0165	gi-15674370	outside	1		nicotine adenine dinucleotide glycohydrolase precursor	positive	x	x	
49	spy0317	gi-15674482	lipoprotein	0	lipoprotein	conserved hypothetical protein	negative		x	
56	spy0385	gi-15674531	lipoprotein	0	lipoprotein	ferrichrome ABC transporter (ferrichrome-binding protein)	positive		x	
63	spy0457	gi-15674576	lipoprotein	0	lipoprotein	putative cyclophilin-type protein	negative		x	
67	spy0714	gi-15674772	outside	0		putative adhesion protein	positive		x	
72	spy0903	gi-15674925	membrane	1		putative ABC transporter (binding protein)	positive	x	x	
78	spy1094	gi-15675078	lipoprotein	0	lipoprotein	conserved hypothetical protein	negative		x	
81	spy1228	gi-15675192	lipoprotein	0	lipoprotein	putative lipoprotein	positive	x	x	x
83	spy1273	gi-15675228	outside	0		CAMP factor	positive		x	

84	spy1274	gi-15675229	lipoprotein	0	lipoprotein	putative amino acid ABC transporter, periplasmic amino acid-binding protein	positive	x	x	
86	spy1294	gi-15675247	lipoprotein	0	lipoprotein	putative maltose/maltodextrin-binding protein	negative		x	
89	spy1390	gi-15675314	lipoprotein	0	lipoprotein	putative protease maturation protein	positive		x	
98	spy1882	gi-15675700	lipoprotein	0	lipoprotein	putative acid phosphatase	positive	x	x	
100	spy2000	gi-15675787	lipoprotein	0	lipoprotein	surface lipoprotein	negative		x	x
103	spy2037	gi-15675810	lipoprotein	0	lipoprotein	conserved hypothetical	positive		x	
157	spy0836	gi-15674871	membrane	1		conserved hypothetical protein	positive		x	
160	spy1113	gi-15675094	membrane	1	RGD	putative acid phosphatase (class B)	positive		x	
177	spy1649	gi-15675521	membrane	1		putative penicillin-binding protein 1a	positive		x	
192	spy2018	gi-15675799	membrane	2	LPXTG	M protein type 1	positive	x		
194	spy2032	gi-15675807	membrane	1		putative ATP-binding cassette transporter-like protein	positive		x	
201	spy2216	gi-15675945	membrane	1		putative serine protease	positive		x	
205	spy0453	gi-15674573	lipoprotein	0	lipoprotein	metal binding protein of ABC transporter	negative	x	x	
284	spy2059	gi-15675827	membrane	1		penicillin-binding protein 2a	ND		x	
286	spy2065	gi-15675830	membrane	1		hypothetical protein	negative		x	
292	spy2073	gi-15675834	membrane	1	RGD	putative endopeptidase Clp ATP-binding chain C	negative	x		

382	spy1842	gi-15675668	outside	1		putative signal peptidase	positive		x	
396	spy2116	gi-15675866	membrane	1		recombination protein	negative	x		
405	spy1028	gi-15675026	membrane	1		putative acetoin dehydrogenase (TPP-dependent) beta chain	positive			x
406	spy1031	gi-15675028	membrane	1		putative dihydrolipoamide dehydrogenase, component E3	positive	x		
500	spy1410	gi-15675330	membrane	2		putative 1-acylglycerol-3-phosphate O-acyltransferase	ND	x		
	spy0047	gi-15674286	cytoplasm			30S ribosomal protein S10	ND		x	
	spy0053	gi-15674291	cytoplasm			30S ribosomal protein S19	ND		x	
	spy0056	gi-15674293	cytoplasm			30S ribosomal protein S3	ND	x		
	spy0063	gi-15674299	cytoplasm			50S ribosomal protein L5	ND	x	x	
	spy0069	gi-15674304	cytoplasm			30S ribosomal protein S5	ND	x		
	spy0098	gi-15674321	cytoplasm			putative DNA-dependent RNA polymerase subunit beta	ND	x		
	spy0127	gi-15674342	cytoplasm			putative signal peptidase I	ND		x	
	spy0274	gi-15674453	cytoplasm			glyceraldehyde-3-phosphate dehydrogenase, plasmin receptor	ND		x	
	spy0611	gi-15674691	cytoplasm			putative translation elongation factor EF-Tu	ND	x		
	spy0666	gi-15674733	cytoplasm			hypothetical protein, phage associated	ND	x		
	spy0686	gi-15674750	cytoplasm			hypothetical protein, phage associated	ND		x	

	spy0688	gi-15674751	cytoplasm			putative major head protein, phage associated	ND		x	
	spy0731	gi-15674785	cytoplasm			putative enolase	ND	x	x	
	spy0913	gi-15674934	cytoplasm			putative ribosomal protein S1-like DNA-binding protein	ND	x		
	spy1200	gi-15675165	membrane			putative signal recognition particle	ND	x		
	spy1281	gi-15675234	membrane			putative signal peptidase I	ND		x	
	spy1721	gi-15675571	cytoplasm			putative initiation factor 2	ND		x	
	spy1750	gi-15675599	cytoplasm			putative malonyl CoA-acyl carrier protein transacylase	ND		x	
	spy1805	gi-15675639	cytoplasm			putative preprotein translocase binding subunit	ND		x	
	spy2070	gi-15675832	cytoplasm			heat shock protein (chaperonin)	ND	x		
	spy2092	gi-15675849	cytoplasm			30S ribosomal protein S2	ND	x	x	
	spy2178	gi-15675914	cytoplasm			30S ribosomal protein S4	ND		x	
		gi-21909751	lipoprotein			oligopeptide permease [Streptococcus pyogenes MGAS315]	ND		x	

Table 9.

	Protein	GAS	Spy	Gene locus (a)	Tryptic peptides (b)	Proteinase K peptides (b)	FACS response (c)
Cell-wall proteins	collagen binding protein			NT01SP0102	-	2	Positive
	hypothetical protein	16	0128	NT01SP0105	-	1	Positive
	putative cell envelope proteinase	57	0416	NT01SP0336	2	3	Positive
	putative extracellular matrix binding protein	68	0737	NT01SP0588	1	-	Positive
	conserved hypothetical protein	143	0747	NT01SP0597	4	2	Positive
	hypothetical protein	158	0843	NT01SP0677	1	-	Positive
	protein GRAB (protein G-related alpha 2M-binding protein)	166	1357	NT01SP1118	2	-	Positive
	hypothetical protein	171	1494	NT01SP1232	-	1	ND
	collagen-like surface protein	188	1983	NT01SP1628	3	2	Positive
	hypothetical protein	190	2009	NT01SP1652	11	35	Positive
	C5A peptidase precursor	191	2010	NT01SP1653	7	16	Positive
	M protein type 1	192	2018	NT01SP1656	26	12	Positive
Lipoproteins	putative ABC transporter	23	0163	NT01SP0136	2	-	Negative
	oligopeptide permease	NS		NT01SP0246	4	-	Negative
	conserved hypothetical protein	49	0317	NT01SP0264	-	1	Positive
	hypothetical protein	685	0319	NT01SP0265	2	-	Positive
	putative cyclophilin-type protein	63	0457	NT01SP0363	1	-	Positive
	hypothetical protein	108	0604	NT01SP0479	1	-	Positive
	putative amino acid ABC transporter, periplasmic amino acid-binding protein	84	1274	NT01SP1051	3	1	Positive
	putative maltose/maltodextrin-binding protein	86	1294	NT01SP1068	5	-	Positive
	putative protease maturation protein	89	1390	NT01SP1145	2	1	Positive
	putative acid phosphatase	98	1882	NT01SP1546	5	3	Positive
	hypothetical protein	103	2037	NT01SP1670	1	-	Positive

Membrane proteins	putative cell division protein (1) (d)	254	0015	NT01SP0014	2	-	Negative
	putative large conductance mechanosensitive channel (1)	286	0780	NT01SP0624	2	-	Positive
	hypothetical protein (1)	287	0802	NT01SP0643	-	1	Negative
	conserved hypothetical protein (1)	288	0836	NT01SP0670	11	1	Positive
	putative ABC transporter (binding protein) (1)	271	0903	NT01SP0728	3	-	Positive
	putative acetoin dehydrogenase (TPP-dependent) beta (1)	320	1028	NT01SP0833	4	1	Positive
	putative dihydrolipoamide dehydrogenase, component E3 (1)	321	1031	NT01SP0836	1	-	Positive
	putative citrate lyase, beta subunit (1)	317	1188	NT01SP0978	1	-	ND
	putative deacetylase (1)	293	1370	NT01SP1129	1	-	ND
	putative cell division protein (1)	307	1520	NT01SP1255	2	2	Positive
	putative beta-galactosidase (1)	308	1586	NT01SP1309	1	-	ND
	hypothetical protein (1)	309	1643	NT01SP1353	1	-	ND
	putative penicillin-binding protein 1a (1)	295	1649	NT01SP1358	2	-	Positive
	hypothetical protein (1)	310	1686	NT01SP1386	3	-	ND
	hypothetical protein sharing similarity with several eukaryotic proteins (1)	311	1798	NT01SP1481	2	-	Positive
	hypothetical protein (1)	312	1939	NT01SP1594	1	-	Positive
	immunogenic secreted protein precursor (1)	300	2025	NT01SP1661	1	-	Negative
	putative ATP-binding cassette transporter-like protein (1)	301	2032	NT01SP1666	1	-	Positive
	hypothetical protein (1)	313	2033	NT01SP1667	3	1	Positive
	mitogenic factor (1)	302	2043	NT01SP1676	1	-	Negative
	putative serine protease (1)	304	2216	NT01SP1817	5	1	Positive
	putative surface exclusion protein (2)	262	0269	NT01SP0226	1	-	Positive
	hypothetical protein (2)	306	1044	NT01SP0849	-	1	ND
	hypothetical protein (2)	291	1154	NT01SP0947	3	1	Positive
	putative 1-acylglycerol-3-phosphate O-acyltransferase (2)	327	1410	NT01SP1162	1	-	ND
	conserved hypothetical protein (2)	303	2184	NT01SP1789	1	-	Negative

	putative glutamine-binding periplasmic protein (3)	323	0277	NT01SP0233	1	-	Positive
	hypothetical protein (3)	264	0351	NT01SP0289	1	-	ND
	putative mannose-specific phosphotransferase system component IID (3)	328	1740	NT01SP1433	1	-	ND
	putative cell-division protein (4)	325	0645	NT01SP0510	1	-	ND
	hypothetical protein (4)	330	1315	NT01SP1085	4	-	Positive
	putative ABC transporter (ATP-binding protein) (4)	331	2029	NT01SP1664	-	1	ND
	putative glycine-betaine binding permease protein (6)	322	0184	NT01SP0154	2	-	Positive
	hypothetical protein (6)	326	0743	NT01SP0593	-	1	ND
	beta-glucoside permease IIABC component (7)	324	0572	NT01SP0454	-	2	ND
	putative L-malate permease (10)	329	1109	NT01SP0906	-	1	ND
	putative integral membrane protein (11)	332	2120	NT01SP1737	1	-	ND
Secreted proteins	putative secreted protein	255	0019	NT01SP0016	2	-	Positive
	nicotine adenine dinucleotide glycohydrolase precursor	260	0165	NT01SP0138	4	2	Positive
	streptolysin O precursor	261	0167	NT01SP0140	2	-	Negative
	putative 42 kDa protein	268	0469	NT01SP0372	4	4	Positive
	putative cyclomaltodextrin glucanotransferase	277	1302	NT01SP1075	1	-	ND
	hypothetical protein	334	1461	NT01SP1204	1	-	Positive
	putative signal peptidase I	318	1842	NT01SP1514	1	-	Positive
	inhibitor of complement-mediated lysis	281	2016	NT01SP1655	1	-	Positive
Cytoplasmic proteins	putative translation elongation factor EF-Tu	346	0611	NT01SP0485	5	5	ND
	50S ribosomal protein L31	350	0717	NT01SP0572	3	-	ND
	conserved hypothetical protein - possibly involved in cell wall localization and side chain formation	352	0792	NT01SP0634	-	1	ND
	50S ribosomal protein L7/L12	355	1073	NT01SP0877	3	-	ND

(a) Gene locus names according to TIGR database (www host server, domain name tigr.org).
(b) Number of peptides identified from trypsin or proteinase K digestions.
(c) A response was considered as positive when the difference between the value of immune serum and that of preimmune serum was higher than 80; otherwise, it was considered as negative.
(d) Number of transmembrane domains predicted by PSORT (in brackets)

Table 10. Hyaluronic acid content of GAS bacteria capsules (fg/CFU)

	M1	M3	M6	M23
1	23,31	49	22,76	26,8
2	14,22	57,51	22,29	19,75
3	14,38	47,63	22,01	21,28
AVERAGE	17,3	51,38	22,35	22,61
SD	5,2	5,35	0,38	3,71

Table 11.

Protein	Gene locus (a)	FACS response (b)	Homology to SF370 gene locus	Found in SF370?
M protein type 3	NT06SP1825	positive	NT01SP1656 (GAS192)	yes
C5A peptidase precursor	NT06SP1824	ND	NT01SP1653 (GAS191)	yes
GRAB precursor	NT06SP0874	positive	NT01SP1118 (GAS166)	yes
protein F2-like protein	NT06SP0112	positive	-	-
hypothetical protein SPs1285	NT06SP1358	negative	NT01SP0677 (GAS158)	yes
putative penicillin binding protein 2X	NT06SP0490	ND	SPY1664 (spy1664)	no
putative large conductance mechanosensitive channel	NT06SP1414	ND	NT01SP0624 (GAS149)	yes
putative 42kDa protein	NT06SP1611	ND	NT01SP0372 (GAS64)	yes
hypothetical protein SPs1270	NT06SP1343	ND	SPY0861 (spy0861)	no
putative translation elongation factor EF-Tu	NT06SP1502	ND	NT01SP0485 (GAS193)	yes

(a) Gene locus names according to TIGR database (www host server, domain name tigr.org).
(b) A response was considered as positive when the difference between the value of immune serum and that of preimmune serum was higher than 80; otherwise, it was considered as negative.

Table 12.

Protein (ref no.)	Immunization route (a)	Challenge (a)	Readout	gene present in SF370	identified (Y/N)
Cell-wall proteins					
M protein (n)	IN	IP	survival	YES	YES
C5a peptidase (n+1)	IN	IN	colonization	YES	YES
hypothetical protein (n+2)	SC	IP	survival	YES	YES
protein GRAB (protein G-related alpha 2M-binding protein) (n+3)	SC	IP	bactericidal assay	YES	YES
SPA (streptococcal protective antigen) (n+4)	IP	IP	survival	NO	-
Sfb I (n+5)	IN	IN	survival	NO	-
FBP54 (n+6)	IN	IP	survival	NO	-
R28 (n+7)	IP	IP	survival	NO	-
Lipoproteins					
ferrichrome ABC transporter (ferrichrome-binding protein) (n+8)	SC		bactericidal assay	YES	NO
putative phosphate ABC transporter (n+8)	SC		bactericidal assay	YES	NO
putative amino acid ABC transporter, periplasmic amino acid-binding protein (n+8)	SC		bactericidal assay	YES	YES
putative protease maturation protein (n+8)	SC		bactericidal assay	YES	YES
hypothetical protein (n+8)	SC		bactericidal assay	YES	NO
Secreted proteins					
SpeA (n+9)	SC	SC	survival	NO	-
SpeB (n+10)	SC	SC	survival	YES	NO

(a). IN: intranasal; SC: subcutaneous; IP: intraperitoneal.

(n) Hu, M.C. et al. Infect Immun 70, 2171-2177 (2002).
(n+1) Ji, Y., Carlson, B., Kondagunta, A. & Cleary, P.P. Infect Immun 65, 2080-2087 (1997).
(n+2) Reid, S.D. et al. J Bacteriol 184, 6316-6324 (2002).
(n+3) McMillan, D.J. et al. Vaccine 22, 2783-2790 (2004).
(n+4) J. B. Dale et al., J. Clin. Investig. 103:1261--1268, 1999
(n+5) Roggiani et al. Infect Immun. 2000 Sep;68(9):5011-7. Toxoids of streptococcal pyrogenic exotoxin A are protective in rabbit models of streptococcal toxic shock syndrome.
(n+6) Kuo et al. Infect Immun. 1998 Aug;66(8):3931-5.
(n+7) Schulze et al. Infect Immun. 2001 Jan;69(1):622-5. Characterization of the domain of fibronectin-binding protein I of Streptococcus pyogenes responsible for elicitation of a protective immune response.
(n+8) Lei et al. J Infect Dis 189, 79-89 (2004).
(n+9) Kawabata et al. Infect Immun. 2001 Feb;69(2):924-30.
(n+10) Stalhammar-Carlemalm et al. Mol Microbiol. 1999 Jul;33(1):208-19.

Table 13.

Protein	Gene locus (a)	FACS response (b)	Homology to SF370 gene locus	Found in SF370?
Cell-wall proteins				
M23 protein	NT03SP1933	positive	NT01SP1656	yes
putative cell envelope proteinase	NT01SP0336	positive	NT01SP0336	yes
hypothetical protein	NT01SP0677	negative	NT01SP0677	yes
C5A peptidase precursor	NT01SP1653	positive	NT01SP1653	yes
GRAB precursor	NT01SP1118	positive	NT01SP1118	yes
Lipoproteins				
putative amino acid ABC transporter, periplasmic amino acid-binding protein	NT01SP1051	positive	NT01SP1051	yes
putative oligopeptidepermease	NT06SP0237	negative	NT01SP0246	yes
putative acid phosphatase	NT01SP1546	negative	NT01SP1546	yes
hypothetical protein SpyM3_0427	NT04SP0510	negative	NT01SP0479	yes
Membrane proteins				
putative ABC transporter (binding protein)	NT01SP0728	negative	NT01SP0728	yes
putative zinc-containing alcohol dehydrogenase	NT01SP0908	ND	NT01SP0908	no
hypothetical protein	NT01SP0643	ND	NT01SP0643	yes
hypothetical protein sharing similarity with several eukaryotic proteins	NT01SP1481	ND	NT01SP1481	yes
conserved hypothetical protein	NT01SP0670	ND	NT01SP0670	yes
Secreted proteins				
putative 42 kDa protein	NT01SP0372	positive	NT01SP0372	yes
putative regulatory protein - RofA related	NT01SP0182	ND	NT01SP0182	no
Cytoplasmic proteins				
elongation factor Tu	NT01SP0485	ND	NT01SP0485	yes

(a) Gene locus names according to TIGR database (www host server, domain name tigr.org).
(b) A response was considered as positive when the difference between the value of immune serum and that of preimmune serum was higher than 80; otherwise, it was considered as negative.

Table 14

Antigen (a)	Mice tested	Survival (%)	Statistical significance (b)
GSTstop	10	0	-
M23 protein (M protein)	8	88	$p < 0.01$
Putative cell envelope proteinase (GAS57)	10	70	$p < 0.01$

(a) GSTstop was considered as negative control; the M23 protein was used as positive control.
(b) Statistical significance was calculated by applying the Student *t*-test.

Table 15.

DOMAIN	2071 (M23)			3348 (M1)			2728 (M12)		
	pre-immune serum	immune serum	delta mean	pre-immune serum	immune serum	delta mean	pre-immune serum	immune serum	delta mean
35d	95.28	105.71	10	172.33	255.69	83	266.79	553.32	287
414d	108.44	175.64	67	201.8	414.32	213	268.91	388.43	120
426d	92.42	136.77	44	145.4	264.04	119	300.53	341.25	41
433d	91.9	111.47	20	168.14	265.3	97	335.2	356.71	22
434d	91.21	93.68	2	139.75	351.4	212	241.07	269.88	29
437d	105.42	109.07	4	218.16	362.96	145	279.21	471.98	193
438d	95.5	130.53	35	166.24	344.14	178	245.95	398.61	153
439d	96.68	112.59	16	178.59	300.51	122	226.79	277.53	51
461d	96.19	88.41	-8	176.06	300.86	125	211.53	450.74	239
465d2	100.53	180.78	80	159.66	549.67	390	188.8	349.13	160
469d	95.98	168.75	73	147.66	298.88	151	213.19	377.33	164
472d	94.03	614.09	520	185.72	314.17	128	271.64	378.72	107
473d	99.02	181.42	82	196.62	301.73	105	195.86	431.12	235
475d	89.2	122.68	33	149.38	585.46	436	190.67	362.94	172
477d	96.6	462.96	366	168.87	174.78	6	255.38	301	46
478d	94.39	154.52	60	212.82	312.16	99	232.82	364.84	132
495d	101.2	247.12	146	189.23	293.31	104	300	281.18	-19
538d	87.54	115.05	28	138.98	398.82	260	195.32	375.33	180
543d	91.8	157.39	66	156.31	313.49	157	287.79	335.98	48
553d	97.15	615.01	518	150.74	407.06	256	204.53	394.8	190
561d	94.97	104.19	9	153.23	354.63	201	183.4	424.87	241
576d	90.59	112.74	22	130.54	205.59	75	222.05	221.83	0

577d2	90.31	127.93	38	160.78	269.23	108	257.02	382.01	125
587d	90.54	110.13	20	137.16	210.82	74	167.69	346.71	179
591d	92.11	106.37	14	155.59	295.09	140	206.83	384.75	178
592d	95.43	100.91	5	152.51	422.63	270	219.72	609.94	390
636d	90.55	147.85	57	139.37	354.74	215	176.56	283.64	107
643d	88.76	113.79	25	161.75	471.95	310	227.82	393.73	166
649d	90.54	106.66	16	147.24	418.67	271	169.2	323.19	154
663d	90.74	131.27	41	184.68	323.92	139	254.16	388.85	135

Table 16. Preferred GAS antigens.

ID_Prot	ID_ORF	Annotation	Gene name	Protein length	Evidence for surface exposure
GAS5	gi-13621340	putative secreted protein	spy0019	398	FACS and association to vesicles
GAS6	gi-13621352	putative choline binding protein	spy0031	374	FACS
GAS15	gi-23503478		NT01SP0102	762	Surface digestion
GAS16p2	gi-13621428	hypothetical protein (fimbrial)	SPy0128	340	Surface digestion
GAS18	gi-13621430	hypothetical protein	spy0130	215	FACS
GAS22	gi-13621454	hypothetical protein	spy0159	292	FACS
GAS23	gi-13621456	putative ABC transporter (lipoprotein)	spy0163	342	FACS and association to membrane vesicles
GAS25	gi-13621460	streptolysin O precursor	spy0167	571	FACS and surface digestion
GAS29	gi-13621499	hypothetical protein	spy0210	410	FACS
GAS30	gi-13621500	exotoxin G precursor	spy0212	234	FACS
GAS36	gi-13622477	putative sugar transporter sugar binding lipoprotei	spy1368	439	FACS

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GAS40a-RR	gi-13621545	putative surface exclusion protein	spy0269	873	Protection (see WO 05/032582) and surface digestion
GAS42	gi-13621559	conserved hypothetical protein	spy0287	420	Protection (see WO 05/032582)
GAS45	gi:19745421	oligopeptide permease presente in M3-M18	NT01SP0246	659	Surface digestion (see also WO 05/032582)
GAS49	gi-13621582	conserved hypothetical protein	spy0317	280	FACS and association to membrane vesicles
GAS56	gi-13621635	ferrichrome ABC transporter (ferrichrome-binding prot)	spy0385	310	Surface digestion and surface exposed domain
GAS57	gi-13621655	putative cell envelope proteinase	spy0416	1647	Surface digestion (see also WO 05/032582)
GAS60	gi-13621668	putative exotoxin (superantigen)	spy0436	232	FACS
GAS62	gi-13622790	conserved hypothetical protein	spy1736	319	FACS
GAS63	gi-13621684	putative cyclophilin-type protein	spy0457	268	FACS and association with membrane vesicles
GAS65	gi-13621895	pyrogenic exotoxin C precursor, phage associated	spy0711	235	FACS
GAS67	gi-13621898	putative adhesion protein	spy0714	515	FACS, surface digestion and association with vesicles
GAS68	gi-13621916	putative extracellular matrix binding protein	spy0737	2045	FACS and surface digestion
GAS69	gi-13621955	putative ABC transporter (substrate-binding protein	spy0778	270	FACS

GAS75	gi-13622180	extracellular hyaluronate lyase	spy1032	805	FACS
GAS76	gi-13622185	conserved hypothetical protein	spy1037	318	FACS
GAS77	gi-13622199	putative collagen-like protein	spy1054	293	FACS
GAS81	gi-13622358	putative lipoprotein	spy1228	350	FACS, surface digestion and association to membrane vesicles
GAS82	gi-13622372	putative phosphate ABC transporter, periplasmic pho	spy1245	288	FACS
GAS84	gi-13622398	putative amino acid ABC transporter, periplasmic am	spy1274	278	Association to membrane vesicles (see also WO 05/032582)
GAS85	gi-13622414	hypothetical protein	spy1290	206	FACS
GAS86	gi-13622418	putative maltose/maltodextrin-binding protein	spy1294	415	FACS and association to membrane vesicles
GAS88	gi-13622470	putative internalin A precursor	spy1361	792	FACS
GAS89	gi-13622493	putative protease maturation protein	spy1390	351	FACS and association to membrane vesicles
GAS91	gi-13622581	conserved hypothetical protein	spy1491	195	FACS
GAS92	gi-13622642	hypothetical protein	spy1558	207	FACS
GAS94	gi-13622705	conserved hypothetical protein	spy1633	535	FACS

GAS95	gi-13622787	putative transcription regulator	spy1733	424	FACS (see also WO 05/032582)
GAS96	gi-13622842	putative ABC transporter (periplasmic binding prot)	spy1795	294	FACS
GAS97	gi-13622846	immunogenic secreted protein precursor homolog	spy1801	503	FACS
GAS98	gi-13622916	putative acid phosphatase	spy1882	284	FACS and association to membrane vesicles
M30098	gi-21909634	putative collagen binding protein (Cpb)	SpyM3_0098	744	See US6777547-B1
GAS99	gi-13622993	streptokinase A precursor	spy1979	440	FACS
GAS100	gi-13623012	surface lipoprotein	spy2000	542	FACS, surface digestion and association to membrane vesicles
M3_0100	gi-21909636	conserved hypothetical protein (fimbrial)	SpyM3_0100	344	
GAS101	gi-13623020	putative laminin adhesion	spy2007	306	FACS
M3_0102	gi-21909638	hypothetical protein	SpyM3_0102	195	
GAS103	gi-13623038	conserved hypothetical	spy2037	309	FACS and association to membrane vesicles
M3_0104	gi-21909640	protein F2 like fibronectin-binding protein	SpyM3_0104	696	See US6355477-B1
GAS105	gi-13623061	putative dipeptidase	spy2066	498	FACS

SPs0106	gi-28895018	protein F2 like fibronectin-binding protein	SPs0106	733	See US6355477-B1
GAS108	gi-13621804	hypothetical protein	spy0604	128	FACS and surface digestion
GAS117-40+A97	gi-15674571	hypothetical protein	spy0448	113	See WO 05/032582
GAS130	gi-13621804	putative protease	spy0604	428	See WO 05/032582
GAS137	gi-13621804	conserved hypothetical protein	spy0604	296	See WO 05/032582
GAS142	gi-13621804	streptolysin S associated ORF	spy0604	352	FACS
GAS143	gi-13621927	conserved hypothetical protein	spy0747	910	FACS and surface digestion
M6_0157	gi-50913503	Fibronectin-binding protein (protein F)	M6_Spy0157	628	See WO 94/01465
GAS158	gi-13621804	hypothetical protein	spy0604	1008	FACS and surface digestion
M6_0159	gi-50913505	Collagen adhesion protein	M6_Spy0159	1037	
GAS159a	gi-13621804	putative spermidine/putrescine ABC transporter	spy0604	357	See WO 05/032582
M6_0160	gi-50913506	Fimbrial structural subunit	M6_Spy0160	557	
GAS165	gi-13622443	conserved hypothetical protein	spy1326	364	FACS

GAS166	gi-13622466	protein GRAB (protein G-related alpha 2M-binding pr	spy1357	217	FACS and surface digestion
GAS175	gi-13622660	3-dehydroquinate synthase	spy1577	357	FACS
GAS178	gi-13622756	hypothetical protein	spy1697	240	FACS
GAS179-1	gi-13622773	putative esterase	spy1718	328	FACS
GAS187	gi-13622989	putative pullulanase	spy1972	1165	FACS
GAS188	gi-13622997	collagen-like surface protei	spy1983	348	FACS and surface digestion
GAS190	gi-13623021	Fba (Fibronectin binding protein)	spy2009	379	FACS and surface digestion
GAS191	gi-15675796	C5a peptidase precursor	spy2010		Surface digestion
GAS193	gi-13623029	immunogenic secreted protein precursor	spy2025	541	Surface digestion (see also WO 05/032582)
GAS195	gi-13623043	mitogenic factor	spy2043	271	FACS and surface digestion
GAS205-1	gi-13621681	metal binding protein of ABC transporter (lipoprote	spy0453	310	FACS and association to membrane vesicles
GAS206	gi-13621912	putative enolase	spy0731	435	FACS and association to membrane vesicles
GAS208	gi-13622029	putative peptidoglycan hydrolase	spy0857	235	FACS

GAS217	gi-13622089	putative oxidoreductase	spy0925	254	See WO 05/032582
GAS218	gi-13622159	putative lysin - phage associated	spy1006	444	FACS
GAS218-t	gi-13622159	putative lysin - phage associated	spy1006	444	FACS
GAS219-1	gi-13622160	streptococcal exotoxin I	spy1007	225	FACS
GAS220	gi-15675016	putative fibronectin binding protein like proteinA	spy1013		
GAS242	gi-13622428	maltose/maltodextrin-binding protein	spy1306	419	FACS
GAS249	gi-13622587	putative hemolysin	spy1497	275	FACS
GAS277a	gi-13622962	hypothetical protein	spy1939	265	Surface digestion (see also WO 05/032582)
GAS290	gi-13622978	conserved hypothetical protein	spy1959	180	See WO 05/032582
GAS294-1	gi-13622306	putative glucose-inhibited division protein	spy1173	448	See WO 05/032582
GAS327	gi-13621729	putative XAA-PRO dipeptidase; X-PRO dipeptidase	spy0513	361	FACS
GAS380	gi-13622855	hypothetical protein	spy1813	995	FACS
GAS384-RR	gi-13622908	putative glycoprotein endopeptidase	spy1874	232	See WO 05/032582

GAS504	gi-13622806	putative trans-2-enoyl-ACP reductase II	spy1751	323	See WO 05/032582
GAS509	gi-13622692	putative O-acetylserine lyase	spy1618	313	See WO 05/032582
GAS511	gi-13622798	putative acetyl-CoA carboxylase alpha subunit	spy1743	256	See WO 05/032582
GAS527	gi-13622332	putative GMP synthase	spy1204	520	See WO 05/032582
GAS529	gi-13622403	putative L-glutamine-D-fructose-6-phosphate amidotr	spy1280	604	See WO 05/032582
GAS533	gi-13622912	putative glutamine synthetase	spy1877	448	See WO 05/032582
GAS680	gi-13621481	conserved hypothetical protein	spy0186	151	numbered GAS58 in WO 05/032582
19224134	gi-19224134	protein F		698	See WO 94/01465
19224135	gi-19224135	Cpa		756	
19224137	gi-19224137	EftLSL.A (fimbrial)		342	
19224141	gi-19224141	protein F2		1161	See US6355477-B1

CLAIMS

1. A composition comprising at least one active agent selected from the group consisting of:

a) a surface-exposed *Streptococcus pyogenes* (GAS) antigen which is shorter by at least one amino acid than a GAS protein and which comprises a surface-exposed domain of the GAS protein, wherein the GAS protein selected from the group consisting of:

(1) GAS proteins listed in Table 2;

(2) GAS proteins listed in Table 3;

(3) GAS proteins listed a table selected from the group consisting of Tables 4A-4R;

(4) GAS proteins listed in Table 5;

(5) GAS proteins listed in Table 6;

(6) GAS proteins listed in Table 7;

(7) GAS proteins listed in Table 8;

(8) GAS proteins listed in Table 9;

(9) GAS proteins listed in Table 11;

(10) GAS proteins listed in Table 12;

(11) GAS proteins listed in Table 13;

(12) GAS proteins listed in Table 14;

(13) GAS proteins listed in Table 15;

(14) GAS proteins listed in Table 16;

(15) GAS4, GAS24, GAS54, GAS63, GAS64, GAS72, GAS86, GAS87, GAS102, GAS149, GAS152, GAS157, GAS163, GAS168, GAS171, GAS177, GAS191, GAS192, GAS194, GAS198, GAS201, GAS224, GAS251, GAS259, GAS262, GAS264, GAS268, GAS282, GAS299, GAS382, GAS405, GAS406, GAS425, GAS433, GAS460, GAS469, GAS493, GAS500, GAS545, GAS558, GAS587, GAS645, GAS650, GAS685, GAS362-1, spy611, spy717, spy792, spy1073, NT01SP0246, and NT01SP0102;

(16) GAS64, GAS149, GAS158, GAS166, GAS191, GAS192, GAS193, SPY1664, and SPY086;

(17) GAS57, GAS64, GAS72, GAS84, GAS98, GAS108, GAS152, GAS157, GAS158, GAS166, GAS191, GAS192, GAS193, GAS268, NT01SP0246, NT01SP0908 (Spy1111), and NT01SP0182 (Spy0216);

(18) GAS64, GAS158, GAS166, GAS191, GAS192, and GAS193;

(19) GAS35, GAS54, GAS70, GAS414, GAS421, GAS425, GAS426, GAS428, GAS433, GAS434, GAS437, GAS438, GAS439, GAS457, GAS461, GAS465, GAS469, GAS472, GAS473, GAS474, GAS475, GAS477, GAS478, GAS486, GAS492, GAS494, GAS495, GAS535, GAS538, GAS540, GAS543, GAS553, GAS560, GAS561, GAS564, GAS565, GAS574, GAS576, GAS577, GAS579, GAS586, GAS587, GAS591, GAS592, GAS607, GAS609, GAS625, GAS626, GAS636, GAS640, GAS643, GAS649, GAS653, GAS657, and GAS663;

(20) GAS35, GAS414, GAS437, GAS438, GAS461, GAS465-2, GAS469, GAS472, GAS473, GAS475, GAS478, GAS495, GAS538, GAS553, GAS561, GAS577-2, GAS591, GAS593, GAS636, GAS643, GAS649, and GAS663;

(21) GAS472, GAS473, and GAS553; and

(22) GAS35, GAS54, GAS70, GAS414, GAS421, GAS425, GAS426, GAS428, GAS433, GAS434, GAS437, GAS438, GAS439, GAS457, GAS461, GAS465, GAS469, GAS472, GAS473, GAS474, GAS475, GAS477, GAS478, GAS486, GAS492, GAS494, GAS495, GAS535, GAS538, GAS540, GAS543, GAS553, GAS560, GAS561, GAS564, GAS565, GAS574, GAS576, GAS577, GAS579, GAS586, GAS587, GAS591, GAS592, GAS607, GAS609, GAS625, GAS626, GAS636, GAS640, GAS643, GAS649, GAS653, GAS657, GAS663, GAS40, GAS5, GAS6, GAS18, GAS22, GAS23, GAS25, GAS29, GAS30, GAS36, GAS39, GAS42, GAS49, GAS56, GAS58, GAS60, GAS62, GAS63, GAS65, GAS67, GAS68, GAS69, GAS74, GAS75, GAS76, GAS77, GAS78, GAS81, GAS82, GAS85, GAS86, GAS89, GAS91, GAS92, GAS93, GAS94, GAS96, GAS97, GAS98, GAS99, GAS100, GAS101, GAS103, GAS104, GAS105, GAS108, GAS117, GAS123, GAS130, GAS131, GAS142, GAS143, GAS158, GAS159, GAS165, GAS166, GAS175, GAS178, GAS179, GAS187, GAS188, GAS190, GAS195, GAS205, GAS206, GAS207, GAS217, GAS218, GAS236, GAS242, GAS249, GAS271, GAS277, GAS290, GAS291, GAS309, GAS327, GAS366, GAS372, GAS380, GAS389, GAS504, GAS509, GAS511,

GAS527, GAS533, GAS685, GAS40/117, GAS117/40, M protein, SagA, Sfb1, and Shp;

(b) a nucleic acid molecule which encodes the GAS antigen; and

(c) an antibody which specifically binds to the GAS antigen.

2. The composition of claim 1 which comprises at least two active agents selected from the group consisting of:

(a) at least two surface-exposed Streptococcus pyogenes (GAS) antigens, wherein a first GAS antigen is expressed on the surface of a first GAS bacterium and a second GAS antigen is expressed on the surface of a second GAS bacterium, wherein the first and second GAS bacteria are different M types;

(b) at least one nucleic acid molecule which encodes the at least two surface-exposed GAS antigens; and

(c) at least two antibodies, wherein a first antibody specifically binds to the first GAS antigen and wherein the second antibody specifically binds to the second GAS antigen.

3. The composition of claim 2 wherein at least one of the first and second GAS bacteria is an M type selected from the group consisting of M1, M3, M6, M11, M12, and M23.

4. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of at least 10 different GAS strains.

5. The composition of claim 4 wherein the at least one GAS antigen is selected from the group consisting of a GAS40 antigen, a GAS5 antigen, a GAS99 antigen, a GAS166 antigen, a GAS96 antigen, a GAS103 antigen, and GAS188 antigen, a GAS76 antigen, a GAS108

antigen, a GAS142 antigen, a GAS190 antigen, a GAS22 antigen, a GAS56 antigen, a GAS77 antigen, and a GAS67 antigen.

6. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of at least seven different M type bacteria.

7. The composition of claim 6 wherein the at least one GAS antigen is selected from the group consisting of a GAS40 antigen, a GAS5 antigen, a GAS99 antigen, a GAS166, GAS96 antigen, a GAS103 antigen, a GAS188 antigen, a GAS76 antigen, a GAS108 antigen, a GAS142 antigen, a GAS190 antigen, a GAS22 antigen, a GAS56 antigen, a GAS77 antigen, and a GAS67 antigen.

8. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of an M1 type GAS bacterium.

9. The composition of claim 8 wherein the at least one GAS antigen is selected from the group consisting of a GAS40 antigen, a GAS5 antigen, a GAS99 antigen, a GAS166 antigen, a GAS96 antigen, a GAS103 antigen, a GAS188 antigen, a GAS76 antigen, a GAS108 antigen, a GAS142 antigen, a GAS190 antigen, a GAS22 antigen, a GAS56 antigen, a GAS77 antigen, a GAS67 antigen, a GAS75 antigen, a GAS93 antigen, a GAS18 antigen, a GAS23 antigen, a GAS69 antigen, a GAS206 antigen, a GAS249 antigen, a GAS123 antigen, a GAS143 antigen, a GAS68 antigen, a GAS25 antigen, a GAS30 antigen, a GAS97 antigen, a GAS105 antigen, a GAS187 antigen, a GAS195 antigen, a GAS242 antigen, a GAS81 antigen, a GAS101 antigen, a GAS6 antigen, a GAS62 antigen, a GAS49 antigen, a GAS89 antigen, a GAS100 antigen, a GAS291 antigen, a GAS98 antigen, a GAS104 antigen, a GAS36 antigen, a GAS158 antigen, a

GAS178 antigen, a GAS175 antigen, a GAS131 antigen, a GAS60 antigen, a GAS380 antigen, and a GAS207 antigen.

10. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of an M3 type GAS bacterium.

11. The composition of claim 10 wherein the at least one GAS antigen is selected from the group consisting of a GAS5 antigen, a GAS99 antigen, a GAS166 antigen, a GAS76 antigen, a GAS22 antigen, a GAS77 antigen, a GAS23 antigen, a GAS69 antigen, a GAS206 antigen, a GAS97 antigen, a GAS81 antigen, a GAS6 antigen, and a GAS78 antigen.

12. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of an M6 type GAS bacterium.

13. The composition of claim 12 wherein the at least one GAS antigen is selected from the group consisting of a GAS5 antigen, a GAS99 antigen, a GAS166 antigen, a GAS96 antigen, a GAS103 antigen, a GAS188 antigen, a GAS76 antigen, a GAS108 antigen, a GAS142 antigen, a GAS190 antigen, a GAS22 antigen, a GAS56 antigen, a GAS77 antigen, a GAS67 antigen, a GAS75 antigen, a GAS93 antigen, a GAS23 antigen, a GAS69 antigen, a GAS206 antigen, a GAS249 antigen, a GAS123 antigen, a GAS143 antigen, a GAS68 antigen, a GAS30 antigen, a GAS97 antigen, a GAS195 antigen, a GAS242 antigen, a GAS81 antigen, a GAS101 antigen, a GAS6 antigen, a GAS62 antigen, a GAS49 antigen, a GAS63 antigen, a GAS85 antigen, a GAS89 antigen, a GAS100 antigen, a GAS179 antigen, a GAS205 antigen, a GAS291 antigen, a GAS98 antigen, a GAS104 antigen, a GAS158 antigen, a GAS178 antigen, a GAS218 antigen, a GAS175 antigen, a GAS78 antigen, a GAS29 antigen, a GAS82 antigen, a GAS165 antigen, a GAS327 antigen, a GAS60 antigen, a GAS271 antigen, and a GAS74 antigen.

14. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of an M11 type GAS bacterium.

15. The composition of claim 14 wherein the at least one GAS antigen is selected from the group consisting of a GAS40 antigen, a GAS99 antigen, a GAS96 antigen, a GAS188 antigen, a GAS142 antigen, a GAS190 antigen, a GAS68 antigen, a GAS25 antigen, and a GAS187 antigen.

16. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of an M12 type GAS bacterium.

17. The composition of claim 16 wherein the at least one GAS antigen is selected from the group consisting of a GAS5 antigen, a GAS99 antigen, a GAS166 antigen, a GAS96 antigen, a GAS103 antigen, a GAS76 antigen, a GAS108 antigen, a GAS142 antigen, a GAS190 antigen, a GAS22 antigen, a GAS77 antigen, a GAS93 antigen, a GAS23 antigen, a GAS69 antigen, a GAS206 antigen, a GAS249 antigen, a GAS123 antigen, a GAS68 antigen, a GAS25 antigen, a GAS30 antigen, a GAS195 antigen, a GAS242 antigen, a GAS6 antigen, a GAS62 antigen, a GAS89 antigen, a GAS179 antigen, a GAS205 antigen, a GAS291 antigen, a GAS98 antigen, a GAS36 antigen, a GAS92 antigen, a GAS218 antigen, a GAS131 antigen, a GAS29 antigen, a GAS91 antigen, a GAS165 antigen, a GAS327 antigen, and a GAS86 antigen.

18. The composition of claim 2 wherein at least one of the first and second GAS antigens is expressed on the surface of an M23 type GAS bacterium.

19. The composition of claim 18 wherein the at least one GAS antigen is selected from the group consisting of a GAS166 antigen, a GAS103 antigen, a GAS142 antigen, a

GAS190 antigen, a GAS67 antigen, a GAS6 antigen, a GAS291 antigen, a GAS29 antigen, and a GAS60 antigen.

20. The composition of any of claims 1-19 wherein the GAS antigen lacks at least one transmembrane domain of the GAS protein.

21. The composition of any of claims 1-20 wherein the first GAS protein contains no transmembrane or cytoplasmic domain of the first GAS protein.

22. The composition of any of claims 1-21 which further comprises a second active agent selected from the group consisting of:

(1) GAS proteins listed in Table 2;

(2) GAS proteins listed in Table 3;

(3) GAS proteins listed a table selected from the group consisting of Tables 4A-4R;

(4) GAS proteins listed in Table 5;

(5) GAS proteins listed in Table 6;

(6) GAS proteins listed in Table 7;

(7) GAS proteins listed in Table 8;

(8) GAS proteins listed in Table 9;

(9) GAS proteins listed in Table 11;

(10) GAS proteins listed in Table 12;

(11) GAS proteins listed in Table 13;

(12) GAS proteins listed in Table 14;

(13) GAS proteins listed in Table 15;

(14) GAS proteins listed in Table 16;

(15) GAS4, GAS24, GAS54, GAS63, GAS64, GAS72, GAS86, GAS87, GAS102, GAS149, GAS152, GAS157, GAS163, GAS168, GAS171, GAS177, GAS191, GAS192, GAS194, GAS198, GAS201, GAS224, GAS251, GAS259, GAS262, GAS264, GAS268, GAS282, GAS299, GAS382, GAS405, GAS406, GAS425, GAS433, GAS460, GAS469, GAS493, GAS500, GAS545, GAS558, GAS587, GAS645, GAS650, GAS685, GAS362-1, spy611, spy717, spy792, spy1073, NT01SP0246, and NT01SP0102;

(16) GAS64, GAS149, GAS158, GAS166, GAS191, GAS192, GAS193, SPY1664, and SPY086;

(17) GAS57, GAS64, GAS72, GAS84, GAS98, GAS108, GAS152, GAS157, GAS158, GAS166, GAS191, GAS192, GAS193, GAS268, NT01SP0246, NT01SP0908 (Spy1111), and NT01SP0182 (Spy0216);

(18) GAS64, GAS158, GAS166, GAS191, GAS192, and GAS193;

(19) GAS35, GAS54, GAS70, GAS414, GAS421, GAS425, GAS426, GAS428, GAS433, GAS434, GAS437, GAS438, GAS439, GAS457, GAS461, GAS465, GAS469, GAS472, GAS473, GAS474, GAS475, GAS477, GAS478, GAS486, GAS492, GAS494, GAS495, GAS535, GAS538, GAS540, GAS543, GAS553, GAS560, GAS561, GAS564, GAS565, GAS574, GAS576, GAS577, GAS579, GAS586, GAS587, GAS591, GAS592, GAS607, GAS609, GAS625, GAS626, GAS636, GAS640, GAS643, GAS649, GAS653, GAS657, and GAS663;

(20) GAS35, GAS414, GAS437, GAS438, GAS461, GAS465-2, GAS469, GAS472, GAS473, GAS475, GAS478, GAS495, GAS538, GAS553, GAS561, GAS577-2, GAS591, GAS593, GAS636, GAS643, GAS649, and GAS663;

(21) GAS472, GAS473, and GAS553; and

(22) GAS35, GAS54, GAS70, GAS414, GAS421, GAS425, GAS426, GAS428, GAS433, GAS434, GAS437, GAS438, GAS439, GAS457, GAS461, GAS465, GAS469, GAS472, GAS473, GAS474, GAS475, GAS477, GAS478, GAS486, GAS492, GAS494, GAS495, GAS535, GAS538, GAS540, GAS543, GAS553, GAS560, GAS561, GAS564, GAS565, GAS574, GAS576, GAS577, GAS579, GAS586, GAS587, GAS591, GAS592, GAS607, GAS609, GAS625, GAS626, GAS636, GAS640, GAS643, GAS649, GAS653, GAS657, GAS663, GAS40, GAS5, GAS6, GAS18, GAS22, GAS23, GAS25, GAS29, GAS30, GAS36, GAS39, GAS42, GAS49, GAS56, GAS58, GAS60, GAS62, GAS63, GAS65, GAS67, GAS68, GAS69, GAS74, GAS75, GAS76, GAS77, GAS78, GAS81, GAS82, GAS85, GAS86, GAS89, GAS91, GAS92, GAS93, GAS94, GAS96, GAS97, GAS98, GAS99, GAS100, GAS101, GAS103, GAS104, GAS105, GAS108, GAS117, GAS123, GAS130, GAS131, GAS142, GAS143, GAS158, GAS159, GAS165, GAS166, GAS175, GAS178, GAS179, GAS187, GAS188, GAS190, GAS195, GAS205, GAS206, GAS207, GAS217, GAS218, GAS236, GAS242, GAS249, GAS271, GAS277, GAS290, GAS291, GAS309, GAS327, GAS366, GAS372, GAS380, GAS389, GAS504, GAS509, GAS511,

GAS527, GAS533, GAS685, GAS40/117, GAS117/40, M protein, SagA, Sfb1, and Shp;

(b) a nucleic acid molecule which encodes the GAS antigen; and

(c) an antibody which specifically binds to the GAS antigen.

23. The composition of any of claims 1-22 wherein at least one of the GAS antigens is a component of a fusion protein.

24. The composition of any of claims 1-22 wherein the GAS protein comprises an amino acid sequence at least 95% identical to an amino acid sequence shown in Table 1.

25. The composition of any of claims 1-22 wherein the GAS protein comprises an amino acid sequence shown in Table 1.

26. The composition of any of claims 1-25 comprising an antigen which is useful in a pediatric vaccine.

27. The composition of any of claims 1-26 comprising an antigen which is useful in a vaccine for elderly or immunocompromised individuals.

28. The composition of any of claims 1-27 further comprising an adjuvant.

29. The composition of any of claims 1-28 wherein the GAS antigen is a polypeptide and the polypeptide is coupled to a carrier protein .

30. The composition of claim 29 wherein the carrier protein is selected from the group consisting of a bacterial toxin, a bacterial toxoid, a N. meningitidis outer membrane protein, a heat shock protein, a pertussis protein, H. influenzae protein D, a cytokine, a lymphokine, a hormone, a growth factor, C. difficile toxin A, C. difficile toxin B, and an iron-uptake protein.

31. A method of making a vaccine for inducing immunity against Streptococcus pyogenes comprising combining the active agent of any of claims 1-30 with a pharmaceutically acceptable carrier,

32. The method of claim 31 wherein the active agent is a polypeptide and the polypeptide is made by a method comprising:

(a) culturing a host cell comprising an expression vector which encodes the polypeptide; and

(b) recovering the polypeptide.

33. Use of at least two surface-exposed Streptococcus pyogenes (GAS) antigens in the manufacture of a medicament for inducing immunity against S. pyogenes infection, wherein a first GAS antigen is expressed on the surface of a first GAS bacterium and a second GAS antigen is expressed on the surface of a second GAS bacterium, wherein the first and second GAS bacteria are different M types.

34. Use of at least one nucleic acid molecule which encodes at least two surface-exposed Streptococcus pyogenes (GAS) antigens in the manufacture of a medicament for inducing immunity against S. pyogenes infection, wherein a first GAS antigen is expressed on the surface of a first GAS bacterium and a second GAS antigen is expressed on the surface of a second GAS bacterium, wherein the first and second GAS bacteria are different M types.

35. Use of at least two antibodies in the manufacture of a medicament for treating S. pyogenes infection, wherein a first antibody specifically binds to a first Streptococcus pyogenes (GAS) antigen expressed on the surface of a first GAS bacterium and wherein a second antibody

specifically binds to a second GAS antigen expressed on the surface of a second GAS bacterium, wherein the first and second GAS bacteria are different M types.

36. Use of an antibody in the manufacture of a medicament for treating S. pyogenes infection, wherein the antibody specifically binds to a surface-exposed GAS antigen which is shorter by at least one amino acid than a first GAS protein and which comprises a surface-exposed domain of the first GAS protein, the first GAS protein selected from the group consisting of GAS protein, the first GAS protein selected from the group consisting of:

(1) GAS proteins listed in Table 2;

(2) GAS proteins listed in Table 3;

(3) GAS proteins listed a table selected from the group consisting of Tables 4A-4R;

(4) GAS proteins listed in Table 5;

(5) GAS proteins listed in Table 6;

(6) GAS proteins listed in Table 7;

(7) GAS proteins listed in Table 8;

(8) GAS proteins listed in Table 9;

(9) GAS proteins listed in Table 11;

(10) GAS proteins listed in Table 12;

(11) GAS proteins listed in Table 13;

(12) GAS proteins listed in Table 14;

(13) GAS proteins listed in Table 15;

(14) GAS proteins listed in Table 16;

(15) GAS4, GAS24, GAS54, GAS63, GAS64, GAS72, GAS86, GAS87, GAS102, GAS149, GAS152, GAS157, GAS163, GAS168, GAS171, GAS177, GAS191, GAS192, GAS194, GAS198, GAS201, GAS224, GAS251, GAS259, GAS262, GAS264, GAS268, GAS282, GAS299, GAS382, GAS405, GAS406, GAS425, GAS433, GAS460, GAS469, GAS493, GAS500, GAS545, GAS558, GAS587, GAS645, GAS650, GAS685, GAS362-1, spy611, spy717, spy792, spy1073, NT01SP0246, and NT01SP0102;

(16) GAS64, GAS149, GAS158, GAS166, GAS191, GAS192, GAS193, SPY1664, and SPY086;

(17) GAS57, GAS64, GAS72, GAS84, GAS98, GAS108, GAS152, GAS157, GAS158, GAS166, GAS191, GAS192, GAS193, GAS268, NT01SP0246, NT01SP0908 (Spy1111), and NT01SP0182 (Spy0216);

(18) GAS64, GAS158, GAS166, GAS191, GAS192, and GAS193;

(19) GAS35, GAS54, GAS70, GAS414, GAS421, GAS425, GAS426, GAS428, GAS433, GAS434, GAS437, GAS438, GAS439, GAS457, GAS461, GAS465, GAS469, GAS472, GAS473, GAS474, GAS475, GAS477, GAS478, GAS486, GAS492, GAS494, GAS495, GAS535, GAS538, GAS540, GAS543, GAS553, GAS560, GAS561, GAS564, GAS565, GAS574, GAS576, GAS577, GAS579, GAS586, GAS587, GAS591, GAS592, GAS607, GAS609, GAS625, GAS626, GAS636, GAS640, GAS643, GAS649, GAS653, GAS657, and GAS663;

(20) GAS35, GAS414, GAS437, GAS438, GAS461, GAS465-2, GAS469, GAS472, GAS473, GAS475, GAS478, GAS495, GAS538, GAS553, GAS561, GAS577-2, GAS591, GAS593, GAS636, GAS643, GAS649, and GAS663;

(21) GAS472, GAS473, and GAS553; and

(22) GAS35, GAS54, GAS70, GAS414, GAS421, GAS425, GAS426, GAS428, GAS433, GAS434, GAS437, GAS438, GAS439, GAS457, GAS461, GAS465, GAS469, GAS472, GAS473, GAS474, GAS475, GAS477, GAS478, GAS486, GAS492, GAS494, GAS495, GAS535, GAS538, GAS540, GAS543, GAS553, GAS560, GAS561, GAS564, GAS565, GAS574, GAS576, GAS577, GAS579, GAS586, GAS587, GAS591, GAS592, GAS607, GAS609, GAS625, GAS626, GAS636, GAS640, GAS643, GAS649, GAS653, GAS657, GAS663, GAS40, GAS5, GAS6, GAS18, GAS22, GAS23, GAS25, GAS29, GAS30, GAS36, GAS39, GAS42, GAS49, GAS56, GAS58, GAS60, GAS62, GAS63, GAS65, GAS67, GAS68, GAS69, GAS74, GAS75, GAS76, GAS77, GAS78, GAS81, GAS82, GAS85, GAS86, GAS89, GAS91, GAS92, GAS93, GAS94, GAS96, GAS97, GAS98, GAS99, GAS100, GAS101, GAS103, GAS104, GAS105, GAS108, GAS117, GAS123, GAS130, GAS131, GAS142, GAS143, GAS158, GAS159, GAS165, GAS166, GAS175, GAS178, GAS179, GAS187, GAS188, GAS190, GAS195, GAS205, GAS206, GAS207, GAS217, GAS218, GAS236, GAS242, GAS249, GAS271, GAS277, GAS290, GAS291, GAS309, GAS327, GAS366, GAS372, GAS380, GAS389, GAS504, GAS509, GAS511,

GAS527, GAS533, GAS685, GAS40/117, GAS117/40, M protein, SagA, Sfb1, and Shp; and

(x) GAS antigens listed in Table 1.

37. A method of inducing immunity against Streptococcus pyogenes comprising administering to an individual an effective amount of any of the compositions of claims 1-30 wherein the active agent is a polypeptide or a nucleic acid molecule.

38. A method of treating a Streptococcus pyogenes infection comprising administering to an individual an effective amount of any of the compositions of claims 1-30 wherein the active agent is an antibody.

39. A kit comprising:

a container comprising the composition of any of claims 1-30; and

instructions for the method of claim 37.

40. A kit comprising:

a container comprising the composition of any of claims 1-30; and

instructions for the method of claim 38.

FIG. 1A

```
       1                                                                50
(SF370) MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M1)
(2634)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M1)
(2580)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M1)
(3280)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M1)
(3348)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M1)
(2913)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M1)

(2726)  MDLEQPKPNQ VKQKIALTST IALLSASVGV SHHVKADDLA PEGAKASNTS (M2)

(2721)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M3)
(3040)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M3)
(3135)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M3)

(2722)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M4)
(2728)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M4)

(4883)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SEETKASNTH (M5)

(2724)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M6)
(2894)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M6)
(3650)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M6)
(5529)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M6)

(2720)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M9)
(2725)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M8)
(4538)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M50)
(5455)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M62)
(5531)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M75)

(3776)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M44)
(5481)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SEETKASNTH (M44)

(4959)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M77)
(D2071) MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M23)
(4436)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M28)
(2727)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M11)
(2719)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M?)
(3789)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M78)
(5476)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M89)
(4088)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M?)

(MANFR) MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M5)
(M8232) MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M18)
(M315)  MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M3)
(SS1)   MDLEQTKPNQ VKQKIALTST IALLSASVGV SHQVKADDRA SGETKASNTH (M3)
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FIG. 1B

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          51                                                        100
(SF370)  DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M1)
(2634)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTKLATALTK TTAEINHLKE (M1)
(2580)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M1)
(3280)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M1)
(3348)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M1)
(2913)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M1)

(2726)   EESLPKTETC EEAKAAVEAV ETNLNQQKAE LTELATALTK TTAEINHLKE (M2)

(2721)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M3)
(3040)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M3)
(3135)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M3)

(2722)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTKLATALTK TTAEINHLKE (M4)
(2728)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M4)

(4883)   DDSLPKPETI QEAKATIEAV EKTLSQQKTK LTELATALTK TTAEINHLKE (M5)

(2724)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M6)
(2894)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M6)
(3650)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M6)
(5529)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M6)

(2720)   DDSLPKPETI QEAKATIEAV EKTLSQQKAK LTELATALTK TTAEINHLKE (M9)
(2725)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINNLKE (M8)
(4538)   DDSLPKPETI QEAKATIEAV EKTLSQQKAK LTELATALTK TTAEINHLKE (M50)
(5455)   DDSLPKPETI QEAKATIEAV EKTLSQQKAK LTELATALTK TTAEINHLKE (M62)
(5531)   DDSLPKPETI QEAKATIEAV EKTLSQQKTK LTELATALTK TTAEINHLKE (M75)

(3776)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M44)
(5481)   DDSLPKPETI QEAKATIEAV EKTLSQQKTK LTELATALTK TTAEINHLKE (M44)

(4959)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M77)
(D2071)  DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTKLATALTK TTAEINHLKE (M23)
(4436)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M28)
(2727)   DDSLPKPETI QEAKATIEAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M11)
(2719)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M?)
(3789)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M78)
(5476)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINNLKE (M89)
(4088)   DDSLPKPETI QEAKATIEAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M?)

(MANFR)  DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M5)
(M8232)  DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M18)
(M315)   DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M3)
(SS1)    DDSLPKPETI QEAKATIDAV EKTLSQQKAE LTELATALTK TTAEINHLKE (M3)
```

FIG. 1C

```
          101                                                       150
(SF370)  QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M1)
(2634)   QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQV (M1)
(2580)   QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M1)
(3280)   QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M1)
(3348)   QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M1)
(2913)   QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M1)
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(2726) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAE**Y**QRELTA TETELHNAQ**V** (M2)

(2721) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M3)
(3040) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M3)
(3135) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M3)

(2722) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M4)
(2728) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M4)

(4883) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M5)

(2724) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M6)
(2894) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M6)
(3650) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M6)
(5529) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M6)

(2720) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M9)
(2725) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M8)
(4538) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M50)
(5455) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M62)
(5531) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M75)

(3776) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M44)
(5481) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M44)

(4959) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M77)
(D2071) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M23)
(4436) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M28)
(2727) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M11)
(2719) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M?)
(3789) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M78)
(5476) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M89)
(4088) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M?)

(MANFR) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M5)
(M8232) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQA (M18)
(M315) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M3)
(SS1) QQDNEQKALT SAQEIYTNTL ASSEETLLAQ GAEHQRELTA TETELHNAQ**V** (M3)

FIG. 1D

```
          151                                                    200
(SF370)   DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M1)
(2634)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M1)
(2580)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M1)
(3280)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M1)
(3348)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M1)
(2913)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M1)

(2726)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M2)

(2721)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M3)
(3040)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M3)
(3135)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M3)

(2722)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M4)
(2728)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M4)

(4883)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M5)

(2724)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M6)
(2894)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M6)
(3650)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M6)
(5529)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M6)

(2720)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M9)
(2725)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M8)
(4538)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M50)
(5455)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M62)
(5531)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMINNPDAI (M75)

(3776)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M44)
(5481)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M44)

(4959)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M77)
(D2071)   DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M23)
(4436)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M28)
(2727)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M11)
(2719)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M?)
(3789)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M78)
(5476)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M89)
(4088)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M?)

(MANFR)   DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M5)
(M8232)   DQHSKETALS EQKASISVET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M18)
(M315)    DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M3)
(SS1)     DQHSKETALS EQKASISAET TRAQDLVEQV KTSEQNIAKL NAMISNPDAI (M3)
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FIG. 1E

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         201                                                  250
(SF370)  TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M1)
(2634)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M1)
(2580)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M1)
(3280)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M1)
(3348)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M1)
(2913)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M1)

(2726)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M2)

(2721)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M3)
(3040)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M3)
(3135)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M3)

(2722)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M4)
(2728)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M4)

(4883)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M5)

(2724)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M6)
(2894)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M6)
(3650)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M6)
(5529)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M6)

(2720)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M9)
(2725)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M8)
(4538)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M50)
(5455)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M62)
(5531)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M75)

(3776)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M44)
(5481)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M44)

(4959)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M77)
(D2071)  TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M23)
(4436)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M28)
(2727)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M11)
(2719)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M?)
(3789)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M78)
(5476)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M89)
(4088)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M?)

(MANFR)  TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M5)
(M8232)  TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M18)
(M315)   TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M3)
(SS1)    TKAAQTANDN TKALSSELEK AKADLENQKA KVKKQLTEEL AAQKAALAEK (M3)
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FIG. 1F

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          251                                                  300
(SF370)  EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M1)
(2634)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M1)
(2580)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M1)
(3280)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M1)
(3348)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M1)
(2913)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M1)

(2726)   EAELSRLKSS APSTQDSIVG TNTMKAPQGY PLEELKKLEA SGYIGSASYN (M2)

(2721)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M3)
(3040)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M3)
(3135)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M3)

(2722)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M4)
(2728)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M4)

(4883)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M5)

(2724)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M6)
(2894)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M6)
(3650)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M6)
(5529)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M6)

(2720)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M9)
(2725)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M8)
(4538)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M50)
(5455)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M62)
(5531)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M75)

(3776)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M44)
(5481)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M44)

(4959)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M77)
D2071)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M23)
(4436)   EAELSRLKSS APSTQDSIVG NNTMKVPQGY PLEELKKLEA SGYIGSASYN (M28)
(2727)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M11)
(2719)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M?)
(3789)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M78)
(5476)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M89)
(4088)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M?)

(MANFR)  EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M5)
(M8232)  EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M18)
(M315)   EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M3)
(SS1)    EAELSRLKSS APSTQDSIVG NNTMKAPQGY PLEELKKLEA SGYIGSASYN (M3)
```

FIG. 1G

	301				350	
(SF370)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M1)
(2634)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M1)
(2580)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M1)
(3280)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M1)
(3348)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M1)
(2913)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M1)
(2726)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	**T**RFVDPDNLT	PEVQNELAQF	(M2)
(2721)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M3)
(3040)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M3)
(3135)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M3)
(2722)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M4)
(2728)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M4)
(4883)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M5)
(2724)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M6)
(2894)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M6)
(3650)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M6)
(5529)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M6)
(2720)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M9)
(2725)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M8)
(4538)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M50)
(5455)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M62)
(5531)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M75)
(3776)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M44)
(5481)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M44)
(4959)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M77)
(D2071)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M23)
(4436)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M28)
(2727)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M11)
(2719)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M?)
(3789)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M78)
(5476)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M89)
(4088)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M?)
(MANFR)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M5)
(M8232)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M18)
(M315)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M3)
(SS1)	NYYKEHADQI	IAKASPGNQL	NQYQDIPADR	NRFVDPDNLT	PEVQNELAQF	(M3)

FIG. 1H

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                                                                    400
(SF370) AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M1)
(2634)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M1)
(2580)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M1)
(3280)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M1)
(3348)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M1)
(2913)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M1)

(2726)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M2)
(2721)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M3)

(3040)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M3)
(3135)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M3)

(2722)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M4)
(2728)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M4)

(4883)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M5)

(2724)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M6)
(2894)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M6)
(3650)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M6)
(5529)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M6)

(2720)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M9)
(2725)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M8)
(4538)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M50)
(5455)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M62)
(5531)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M75)

(3776)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M44)
(5481)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M44)

(4959)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M77)
(D2071) AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M23)
(4436)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M28)
(2727)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M11)
(2719)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M?)
(3789)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M78)
(5476)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M89)
(4088)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M?)

(MANFR) AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M5)
(M8232) AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M18)
(M315)  AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M3)
(SS1)   AAHMINSVRR QLGLPPVTVT AGSQEFARLL STSYKKTHGN TRPSFVYGQP (M3)
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FIG. 1I

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                                                                    450
(SF370)  GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M1)
(2634)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M1)
(2580)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M1)
(3280)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M1)
(3348)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M1)
(2913)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M1)

(2726)   GASGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M2)

(2721)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M3)
(3040)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M3)
(3135)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M3)

(2722)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M4)
(2728)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M4)

(4883)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M5)

(2724)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M6)
(2894)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M6)
(3650)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M6)
(5529)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M6)

(2720)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M9)
(2725)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M8)
(4538)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M50)
(5455)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M62)
(5531)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M75)

(3776)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M44)
(5481)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M44)

(4959)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M77)
(D2071)  GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M23)
(4436)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M28)
(2727)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M11)
(2719)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M?)
(3789)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M78)
(5476)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M89)
(4088)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M?)

(MANFR)  GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M5)
(M8232)  GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M18)
(M315)   GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M3)
(SS1)    GVSGHYGVGP HDKTIIEDSA GASGLIRNDD NMYENIGAFN DVHTVNGIKR (M3)
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FIG. 1J

FC451

500

(SF370)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M1)
(2634)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M1)
(2580)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M1)
(3280)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M1)
(3348)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M1)
(2913)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M1)
(2726)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M2)
(2721)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M3)
(3040)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M3)
(3135)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M3)
(2722)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M4)
(2728)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M4)
(4883)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M5)
(2724)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M6)
(2894)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M6)
(3650)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M6)
(5529)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M6)
(2720)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M9)
(2725)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M8)
(4538)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNP**K**APVYL	GFSTSNVGSL	(M50)
(5455)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNP**K**APVYL	GFSTSNVGSL	(M62)
(5531)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNP**K**APVYL	GFSTSNVGSL	(M75)
(3776)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M44)
(5481)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M44)
(4959)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M77)
(D2071)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M23)
(4436)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M28)
(2727)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M11)
(2719)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M?)
(3789)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M78)
(5476)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M89)
(4088)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M?)
(MANFR)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	K**R**NPNAPVYL	GFSTSNVGSL	(M5)
(M8232)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNP**K**APVYL	GFSTSNVGSL	(M18)
(M315)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M3)
(SS1)	GIYDSIKYML	FTDHLHGNTY	GHAINFLRVD	KHNPNAPVYL	GFSTSNVGSL	(M3)

FIG. 1K

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          501                                                  550
(SF370)  NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M1)
(2634)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M1)
(2580)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M1)
(3280)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M1)
(3348)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M1)
(2913)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M1)

(2726)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M2)

(2721)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M3)
(3040)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M3)
(3135)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M3)

(2722)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M4)
(2728)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M4)

(4883)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M5)

(2724)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M6)
(2894)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M6)
(3650)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M6)
(5529)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M6)

(2720)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M9)
(2725)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M8)
(4538)   NEHFVMFPES NIANHQRFNK TPIKTVGSTK DYAQRVGTVS DTIAAIKGKV (M50)
(5455)   NEHFVMFPES NIANHQRFNK TPIKTVGSTK DYAQRVGTVS DTIAAIKGKV (M62)
(5531)   NEHFVMFPES NIANHQRFNK TPIKTVGSTK DYAQRVGTVS DTIAAIKGKV (M75)

(3776)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M44)
(5481)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M44)

(4959)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M77)
(D2071)  NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M23)
(4436)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M28)
(2727)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVSTVS DTIAAIKGKV (M11)
(2719)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M?)
(3789)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M78)
(5476)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M89)
(4088)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVSTVS DTIAAIKGKV (M?)

(MANFR)  NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M5)
(M8232)  NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M18)
(M315)   NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M3)
(SS1)    NEHFVMFPES NIANHQRFNK TPIKAVGSTK DYAQRVGTVS DTIAAIKGKV (M3)
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FIG. 1T.

	551					600	
(SF370)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M1)
(2634)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M1)
(2580)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M1)
(3280)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M1)
(3348)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M1)
(2913)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M1)
(2726)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M2)
(2721)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M3)
(3040)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M3)
(3135)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M3)
(2722)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M4)
(2728)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M4)
(4883)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M5)
(2724)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M6)
(2894)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M6)
(3650)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M6)
(5529)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M6)
(2720)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M9)
(2725)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M8)
(4538)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M50)
(5455)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M62)
(5531)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M75)
(3776)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M44)
(5481)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M44)
(4959)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M77)
(D2071)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M23)
(4436)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M28)
(2727)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M11)
(2719)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M?)
(3789)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M78)
(5476)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M89)
(4088)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M?)
(MANFR)	SSLENRLSAI	HQEADIMAAQ	AKVSQL**E**GKL	ASTLKQSDSL	NLQVRQLNDT		(M5)
(M8232)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M18)
(M315)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M3)
(SS1)	SSLENRLSAI	HQEADIMAAQ	AKVSQLQGKL	ASTLKQSDSL	NLQVRQLNDT		(M3)

FIG. 1M

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          601                                                 650
(SF370)   KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M1)
(2634)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M1)
(2580)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M1)
(3280)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M1)
(3348)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M1)
(2913)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M1)

(2726)    KGSLRTELLV AKAKQAQLEA TRDQSLAKLA SLKAAMHQTK ALAEQAAARV (M2)

(2721)    KGSLRTELLV AKAKQAQLEA TRDQSLAKLA SLKAAMHQTK ALAEQAAARV (M3)
(3040)    KGSLRTELLV AKAKQAQLEA TRDQSLAKLA SLKAAMHQTK ALAEQAAARV (M3)
(3135)    KGSLRTELLV AKAKQAQLEA TRDQSLAKLA SLKAAMHQTK ALAEQAAARV (M3)

(2722)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M4)
(2728)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M4)

(4883)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M5)

(2724)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M6)
(2894)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M6)
(3650)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M6)
(5529)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M6)

(2720)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M9)
(2725)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M8)
(4538)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M50)
(5455)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M62)
(5531)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M75)

(3776)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M44)
(5481)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M44)

(4959)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M77)
(D2071)   KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M23)
(4436)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE VLAEQAAARV (M28)
(2727)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M11)
(2719)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M?)
(3789)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M78)
(5476)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M89)
(4088)    KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M?)

(MANFR)   KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M5)
(M8232)   KGSLRTELLA AKAKQAQLEA TRDQSLAKLA SLKAALHQTE ALAEQAAARV (M18)
(M315)    KGSLRTELLV AKAKQAQLEA TRDQSLAKLA SLKAAMHQTK ALAEQAAARV (M3)
(SS1)     KGSLRTELLV AKAKQAQLEA TRDQSLAKLA SLKAAMHQTK ALAEQAAARV (M3)
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FIG. 1N

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                 651                                                700
(SF370)  TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(2634)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNIKQDLAKT TSSLLNAQET (M1)
(2580)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(3280)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(3348)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(2913)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)

(2726)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQET (M2)

(2721)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M3)
(3040)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M3)
(3135)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M3)

(2722)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M4)
(2728)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M4)

(4883)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M5)

(2724)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M6)
(2894)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M6)
(3650)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M6)
(5529)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M6)

(2720)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M9)
(2725)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQET (M8)
(4538)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQET (M50)
(5455)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQET (M62)
(5531)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQET (M75)

(3776)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M44)
(5481)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M44)

(4959)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M77)
(D2071)  TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M23)
(4436)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQET (M28)
(2727)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M11)
(2719)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M?)
(3789)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M78)
(5476)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M89)
(4088)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M?)

(MANFR)  TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(M8232)  TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(M315)   TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
(SS1)    TALVAKKAHL QYLRDFKLNP NRLQVIRERI DNTKQDLAKT TSSLLNAQEA (M1)
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        701                                                     750
(SF370) LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M1)
(2634)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M1)
(2580)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M1)
(3280)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M1)
(3348)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M1)
(2913)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M1)

(2726)  LAALQAKKSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M2)

(2721)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M3)
(3040)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M3)
(3135)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M3)

(2722)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M4)
(2728)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M4)

(4883)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M5)

(2724)  LAALQAKQSS LEATIATTEH QLTLLKILAN EKEYRHLDED IATVPDLQVA (M6)
(2894)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M6)
(3650)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M6)
(5529)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M6)

(2720)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M9)
(2725)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M8)
(4538)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M50)
(5455)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M62)
(5531)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M75)

(3776)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M44)
(5481)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M44)

(4959)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M77)
(D2071) LAALQAKQSS LEATIATTEH QLTLLKTLAN ENEYRHLDED IATVPDLQVA (M23)
(4436)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKGYRHLDED IATVPDLQVA (M28)
(2727)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M11)
(2719)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M?)
(3789)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M78)
(5476)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M89)
(4088)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M?)

(MANFR) LAVLQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M5)
(M8232) LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M18)
(M315)  LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M3)
(SS1)   LAALQAKQSS LEATIATTEH QLTLLKTLAN EKEYRHLDED IATVPDLQVA (M3)
```

FIG. 1P

(SF370)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M1)
(2634)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M1)
(2580)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M1)
(3280)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M1)
(3348)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M1)
(2913)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M1)
(2726)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M2)
(2721)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M3)
(3040)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M3)
(3135)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M3)
(2722)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M4)
(2728)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETK**H**L LEASARLAAE NTSLVAEALV	(M4)
(4883)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M5)
(2724)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M6)
(2894)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M6)
(3650)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M6)
(5529)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M6)
(2720)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M9)
(2725)	P**S**LTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M8)
(4538)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M50)
(5455)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M62)
(5531)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M75)
(3776)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M44)
(5481)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M44)
(4959)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M77)
(D2071)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M23)
(4436)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M28)
(2727)	P**S**LTGVKLLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M11)
(2719)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M?)
(3789)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M78)
(5476)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M89)
(4088)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M?)
(MANFR)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M5)
(M8232)	PPLTGVKPLS YSKIDTTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M18)
(M315)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M3)
(SS1)	P**S**LTGVKPLS YSK**VE**TTPLV QEMVKETKQL LEASARLAAE NTSLVAEALV	(M3)

FIG. 1Q

	801				850	
(SF370)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M1)
(2634)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M1)
(2580)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M1)
(3280)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M1)
(3348)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M1)
(2913)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M1)
(2726)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SD**I**DESTQRA	(M2)
(2721)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M3)
(3040)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M3)
(3135)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M3)
(2722)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M4)
(2728)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M4)
(4883)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M5)
(2724)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M6)
(2894)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M6)
(3650)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M6)
(5529)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M6)
(2720)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M9)
(2725)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M8)
(4538)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M50)
(5455)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M62)
(5531)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M75)
(3776)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M44)
(5481)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M44)
(4959)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M77)
(D2071)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSY**D**	SGSSTTSNLI	SDVDESTQRA	(M23)
(4436)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M28)
(2727)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M11)
(2719)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M?)
(3789)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M78)
(5476)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M89)
(4088)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M?)
(MANFR)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M5)
(M8232)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M18)
(M315)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M3)
(SS1)	GQTSEMVASN	AIVSKITSSI	TQPSSKTSYG	SGSSTTSNLI	SDVDESTQRA	(M3)

FIG. 1R

	851		873	
(SF370)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M1)
(2634)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M1)
(2580)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M1)
(3280)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M1)
(3348)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M1)
(2913)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M1)
(2726)	LKAGVVMLAA	VGLTG**VKL**RK	**DT**K	(M2)
(2721)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M3)
(3040)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M3)
(3135)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M3)
(2722)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M4)
(2728)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M4)
(4883)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M5)
(2724)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M6)
(2894)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M6)
(3650)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M6)
(5529)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M6)
(2720)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M9)
(2725)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M8)
(4538)	LKAGVVMLAA	**I**GLTGFRFRK	ESK	(M50)
(5455)	LKAGVVMLAA	**I**GLTGFRFRK	ESK	(M62)
(5531)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M75)
(3776)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M44)
(5481)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M44)
(4959)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M77)
(D2071)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M23)
(4436)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M28)
(2727)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M11)
(2719)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M?)
(3789)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M78)
(5476)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M89)
(4088)	LKAGVVMLAA	VGLTGFRFRK	ESK	(M?)
(MANFR)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M5)
(M8232)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M18)
(M315)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M3)
(SS1)	LKAGVVMLAA	VGLTGFRFRK	ES**R**	(M3)

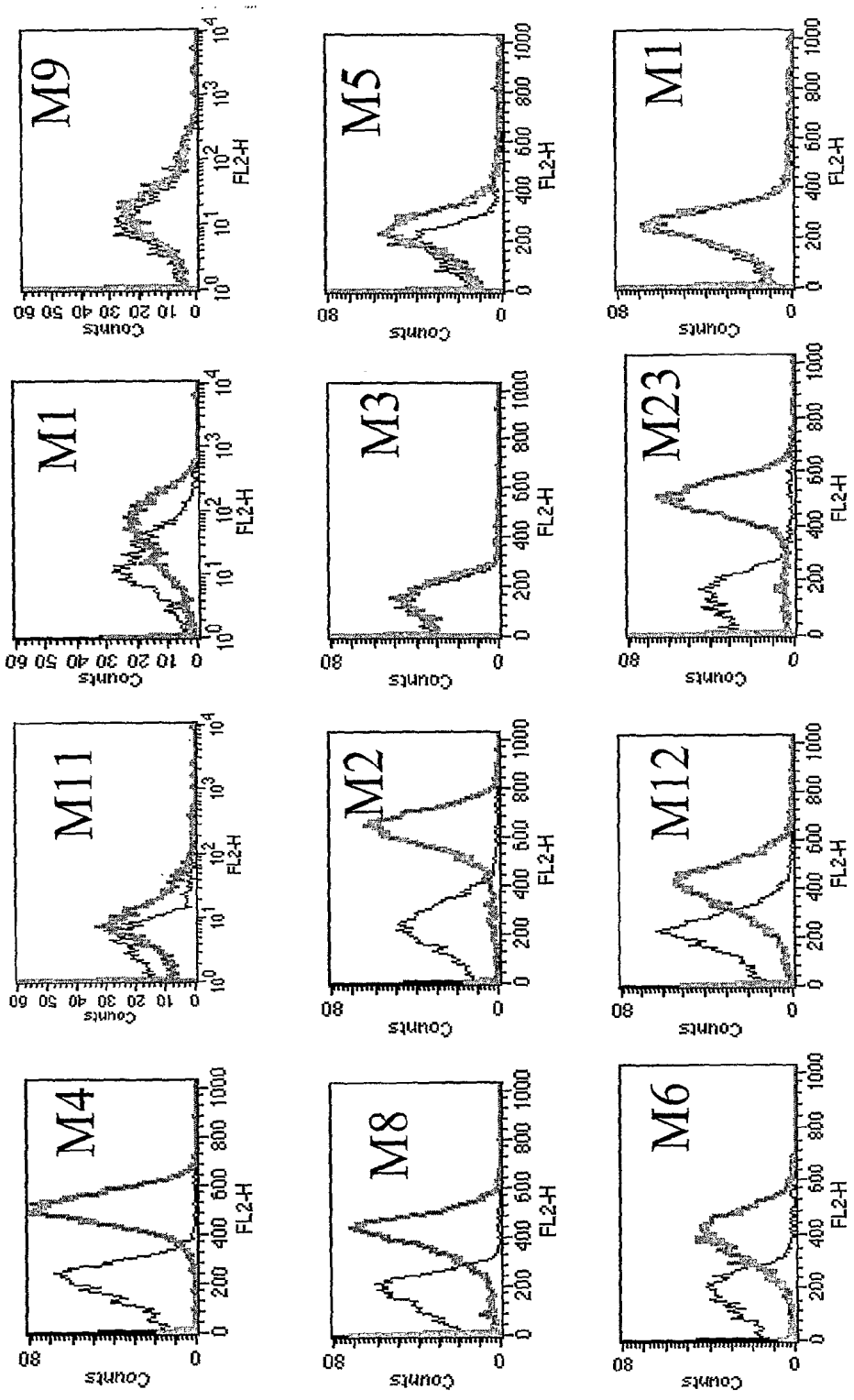
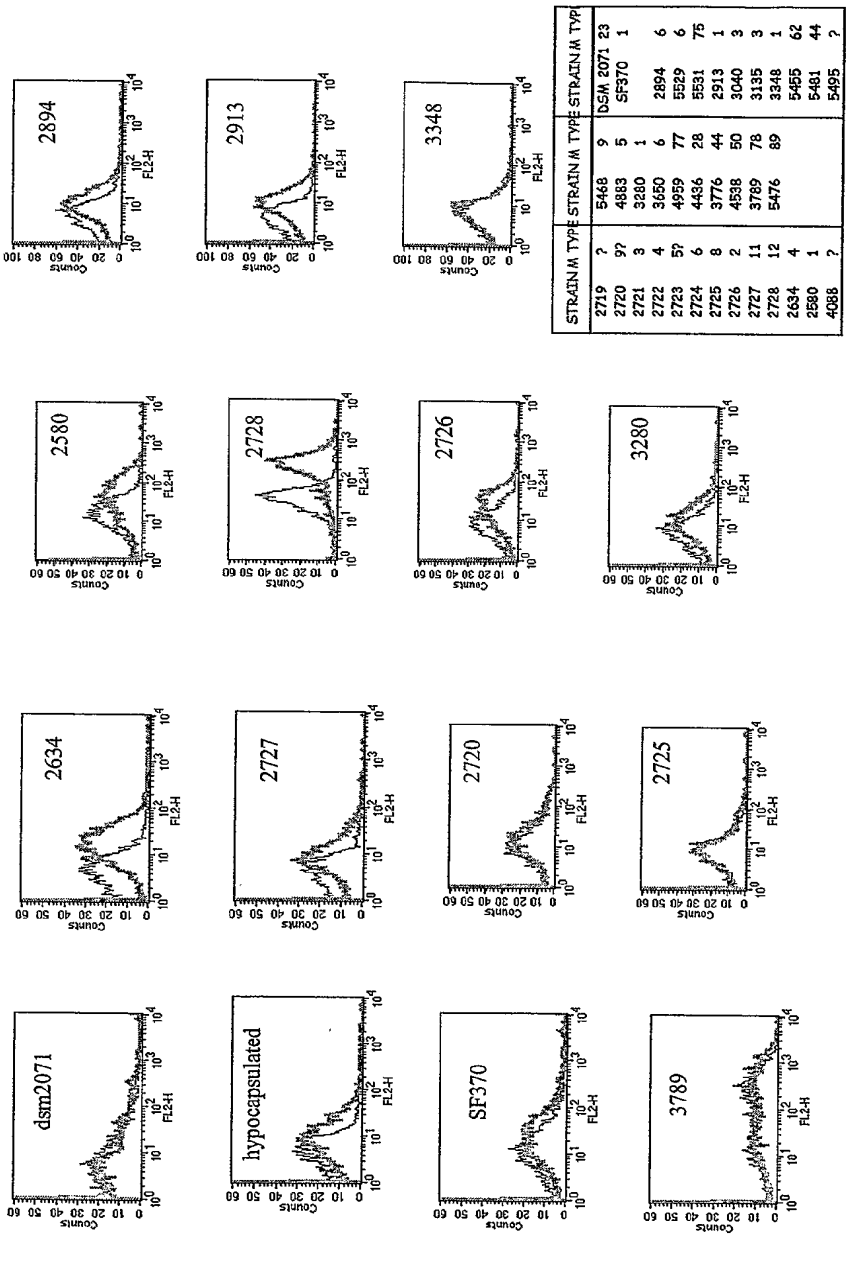


FIG. 2

FIG. 3



STRAIN	M TYPE	STRAIN	M TYPE	STRAIN	M TYPE
2719	?	5468	9	DSM 2071	23
2720	97	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	52	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5455	62
2580	1			5481	44
4088	?			5495	?

FIG. 4A

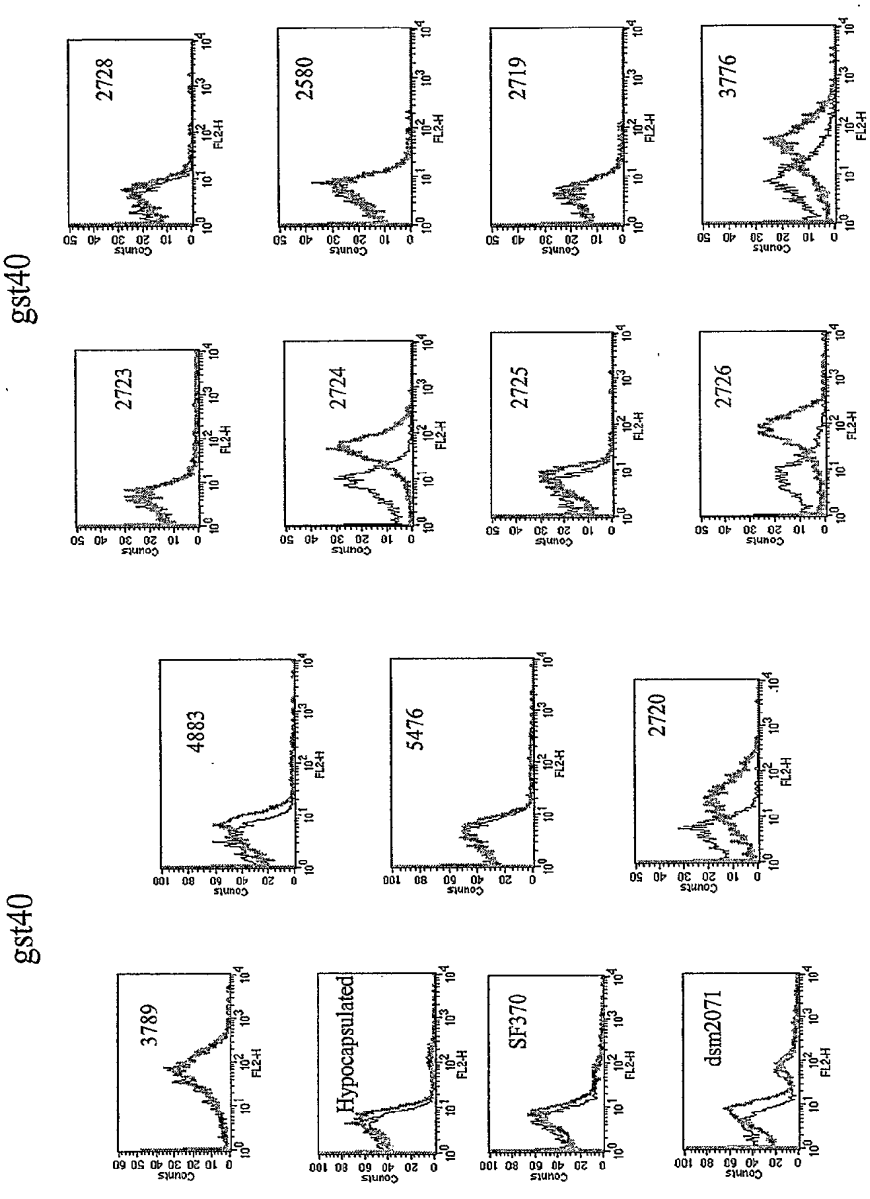
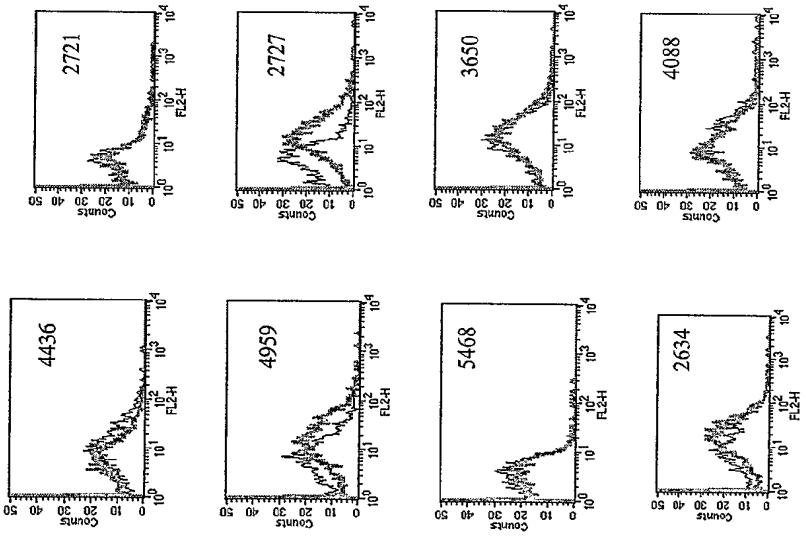
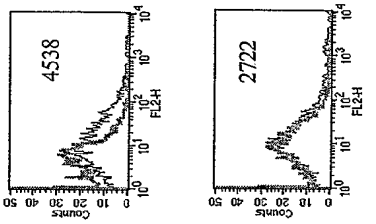

gst40
2728
2580
2719
3776
2723
2724
2725
2726
gst40
4883
5476
2720
3789
Hypocapsulated
SF370
dsm2071
Counts
FL2-H

FIG. 4B





STRAIN	M TYPE	STRAIN	M TYPE	STRAIN	M TYP
2719	?	5468	9	DSM 2071	23
2720	9?	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	5?	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5455	62
2580	1			5481	44
4088	?			5495	?

FIG. 5A

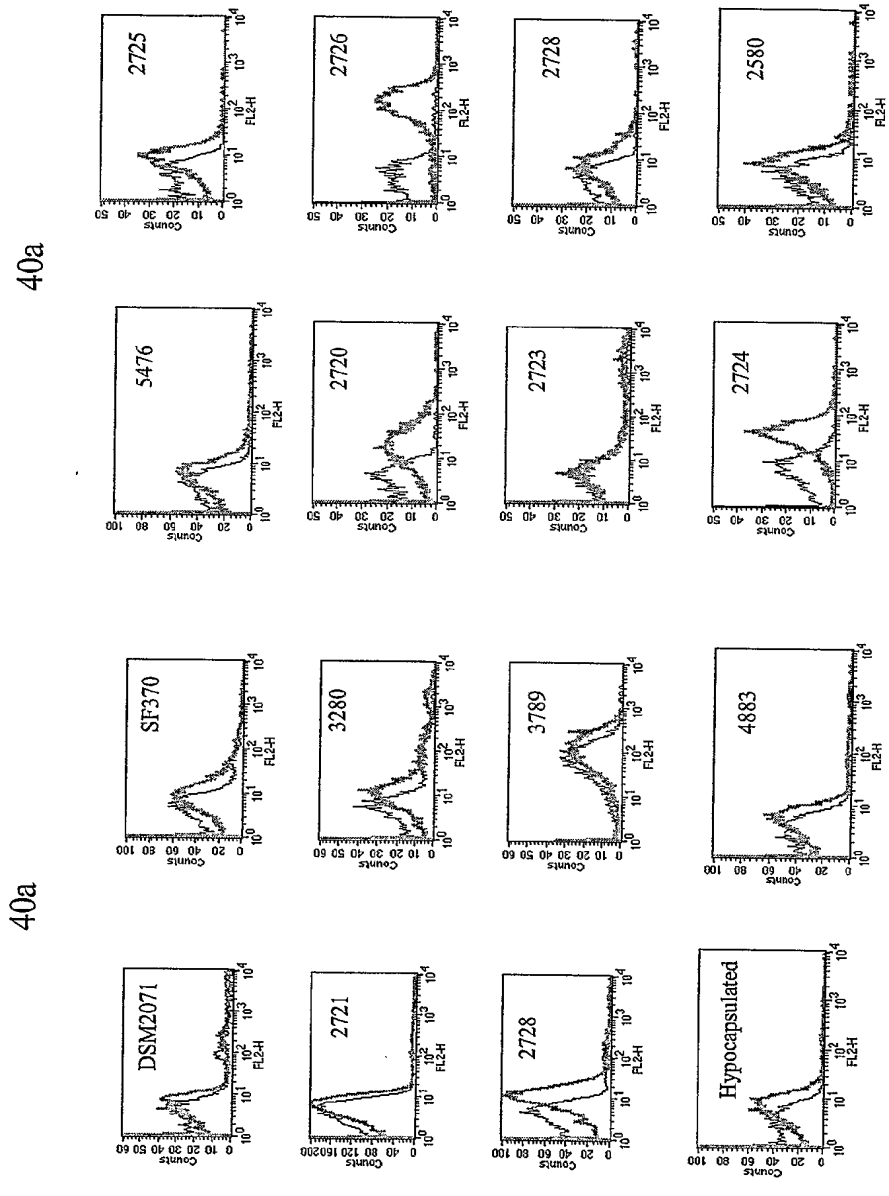
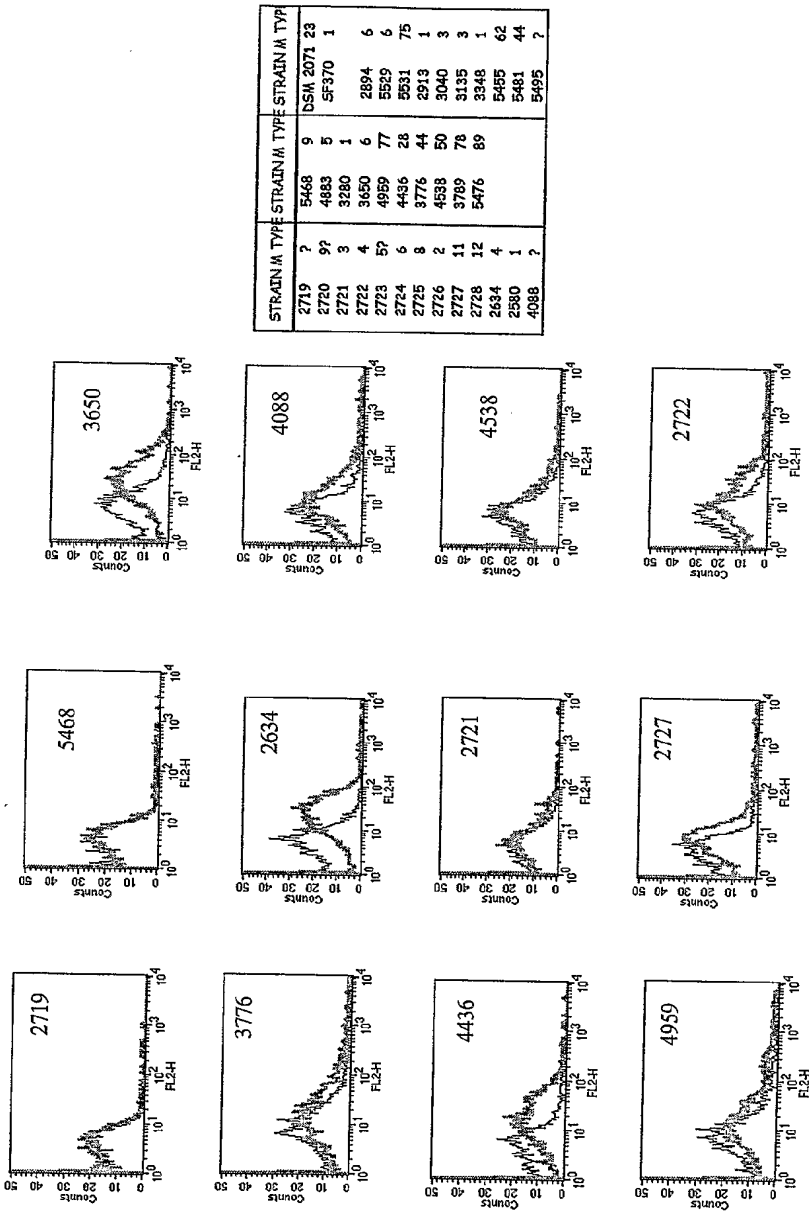

40a
40a
DSM2071
2721
2728
Hypocapsulated
SF370
3280
3789
4883
5476
2720
2723
2724
2725
2726
2728
2580
Counts
FL2-H

FIG. 5B



STRAIN #	TYPE	STRAIN #	TYPE	STRAIN #	TYPE
2719	?	5468	9	DSM 2071	23
2720	??	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	??	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5485	62
2580	1			5481	44
4088	?			5495	?

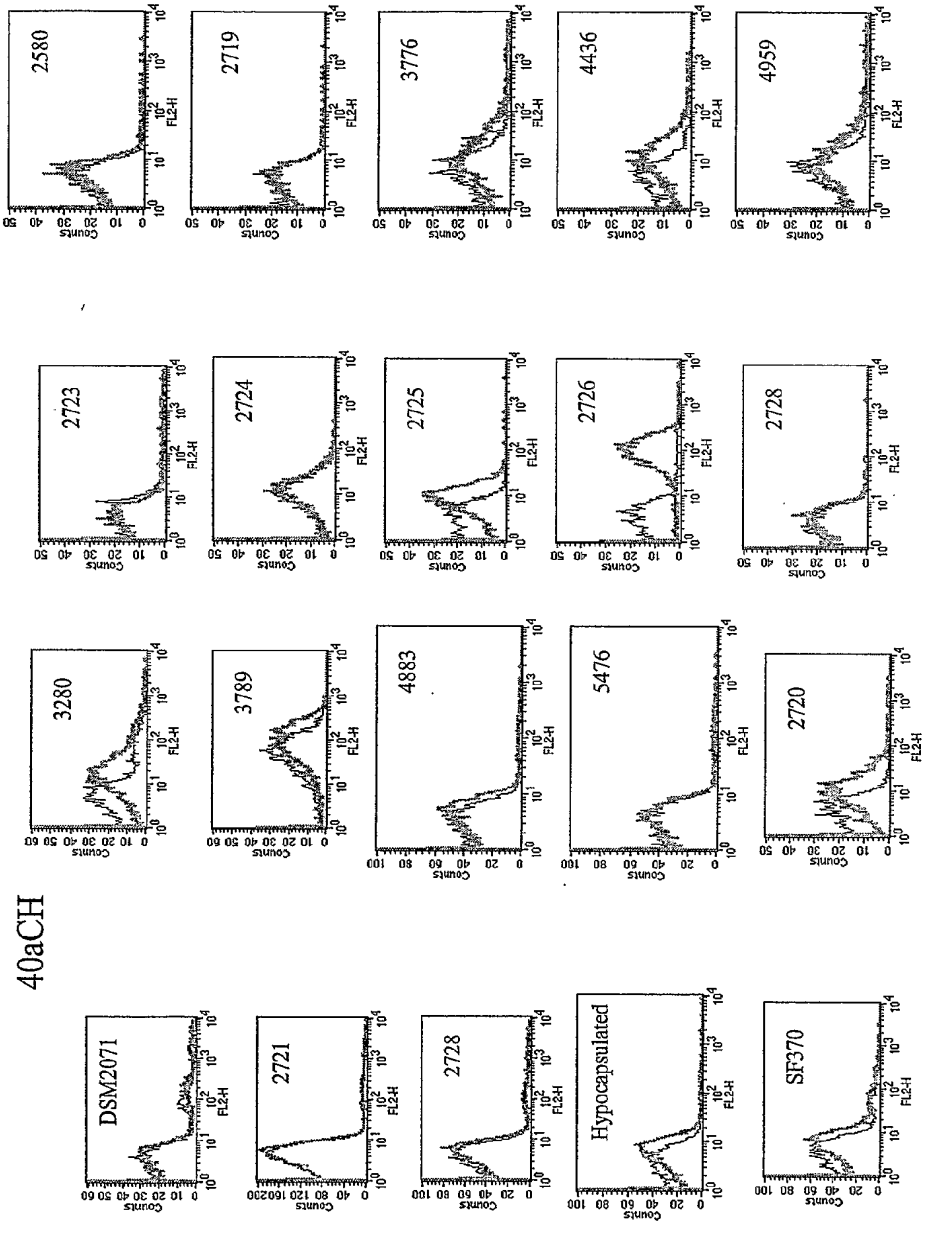
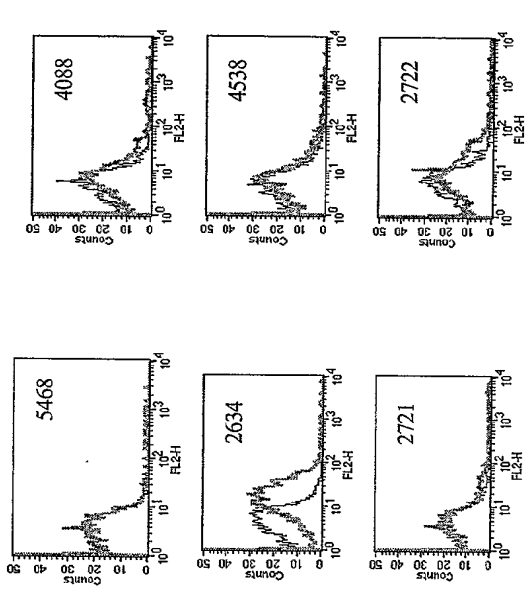

40aCH
DSM2071
2721
2728
Hypocapsulated
SF370
3280
3789
4883
5476
2720
2723
2724
2725
2726
2728
2580
2719
3776
4436
4959
Counts
FL2-H

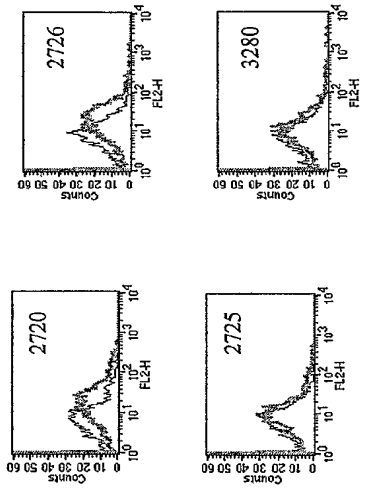
FIG. 6A



STRAIN #	TYPE	STRAIN #	TYPE	STRAIN #	TYP
2719	?	5468	9	DSM 2071	23
2720	9?	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	5?	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5455	62
2580	1			5481	44
4088	?			5495	?

FIG. 6B

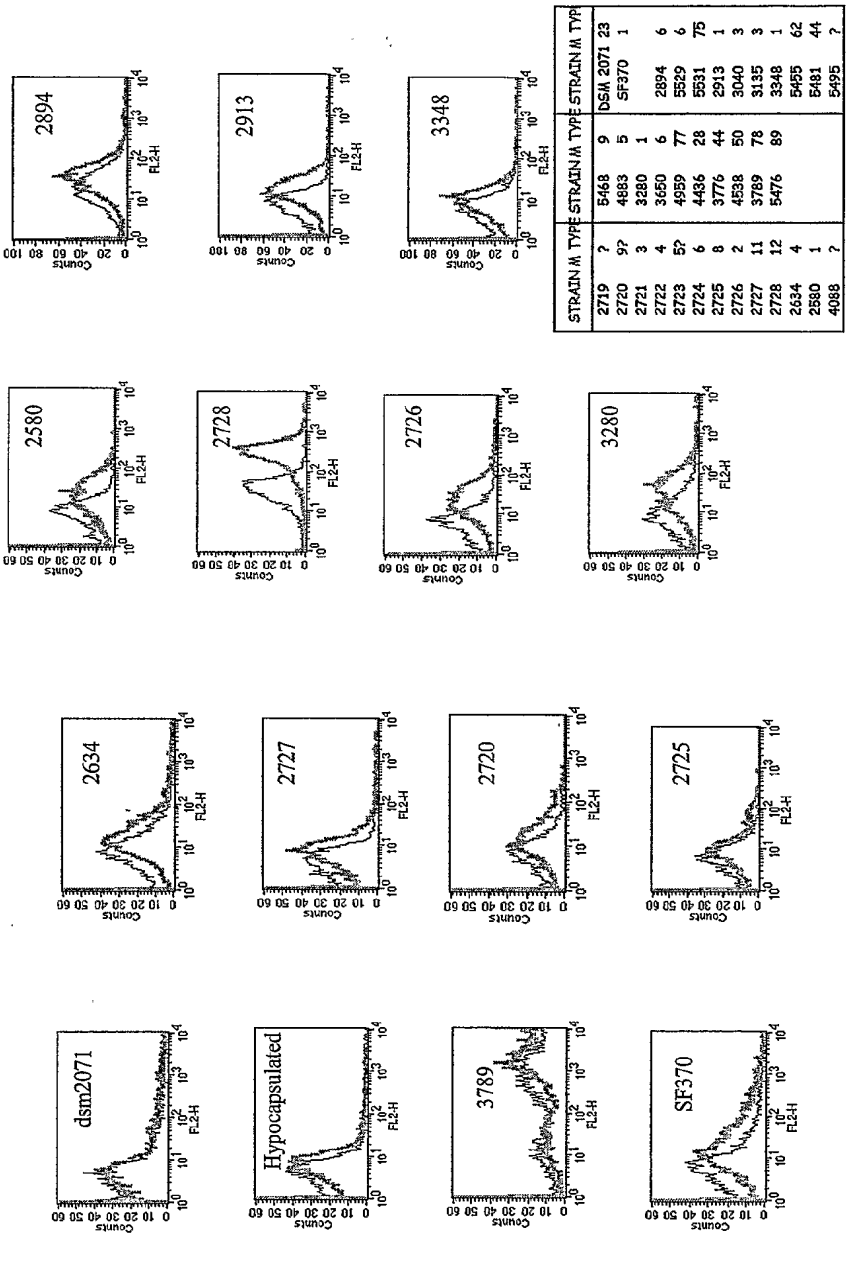
40/117



STRAIN #	TYPE	STRAIN #	TYPE	STRAIN #	TYP
2719	?	5468	9	DSM 2071	23
2720	9?	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	5?	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5465	62
2580	1			5481	44
4088	?			5495	?

FIG. 7

FIG. 8



STRAIN	M. TYPE	STRAIN	M. TYPE	STRAIN	M. TYP
2719	?	5468	9	DSM 2071	23
2720	9?	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	5?	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5455	62
2580	1			5481	44
4088	?			5495	?

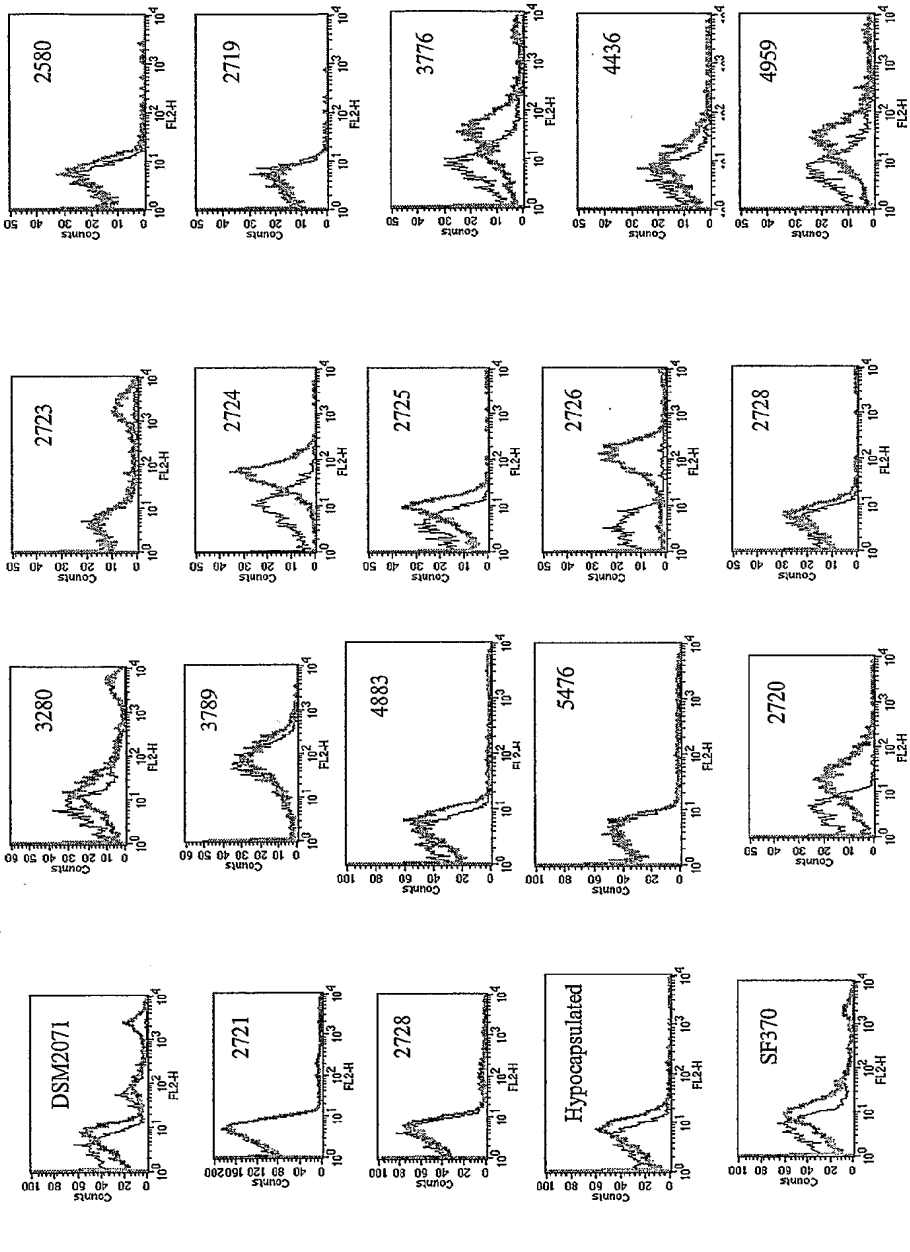
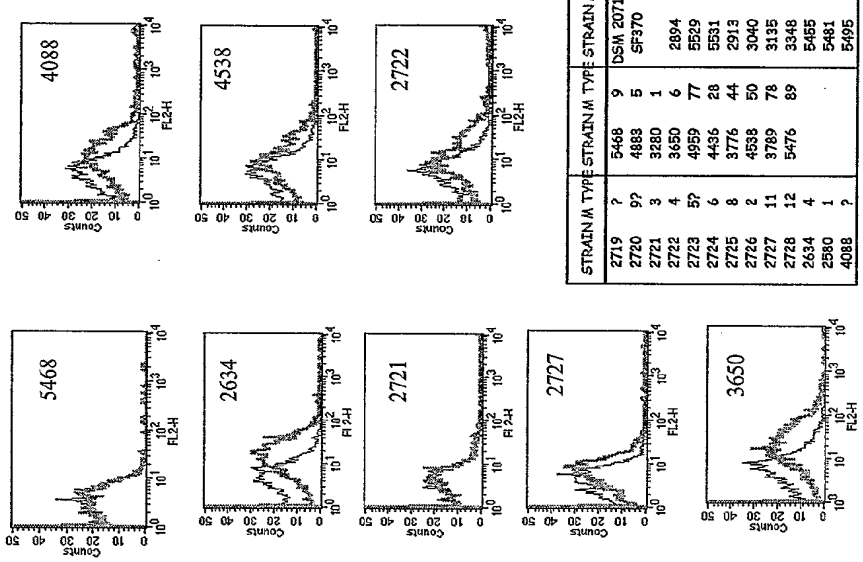

2580
2719
3776
4436
4959
2723
2724
2725
2726
2728
3280
3789
4883
5476
2720
DSM2071
2721
2728
Hypocapsulated
SF370
Counts
FL2-H

FIG. 9A
40aRR



STRAIN M TYPE	?	STRAIN M TYPE	STRAIN M TYPE	STRAIN M TYPE	
2719	?	5468	9	DSM 2071	23
2720	99	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	57	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5465	62
2580	1			5481	44
4088	?			5495	?

FIG. 9B
40aRR

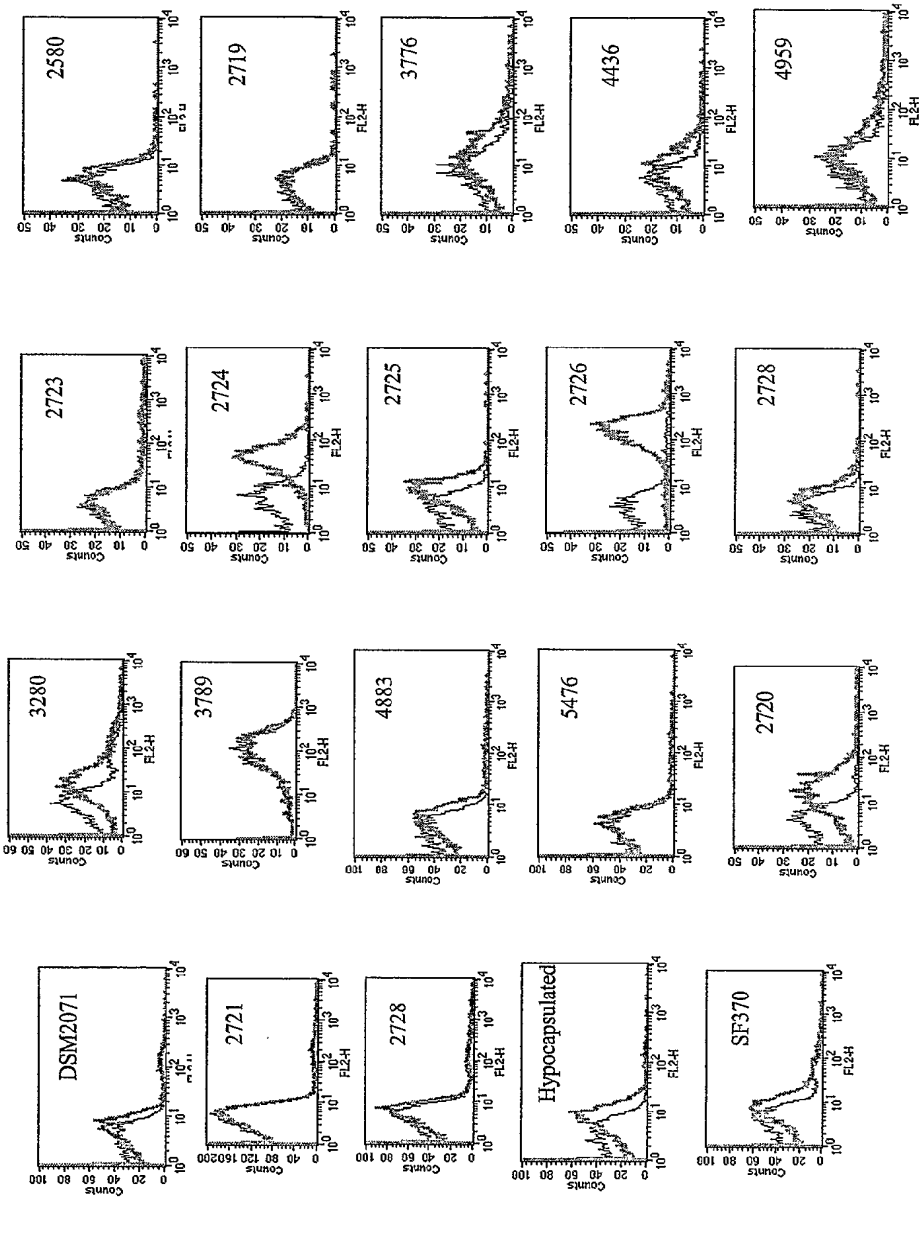
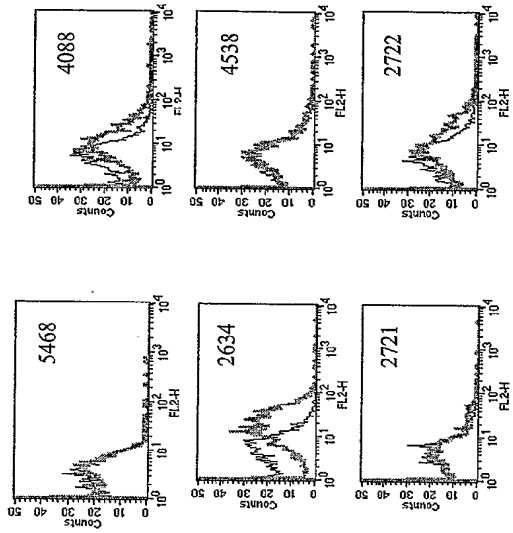


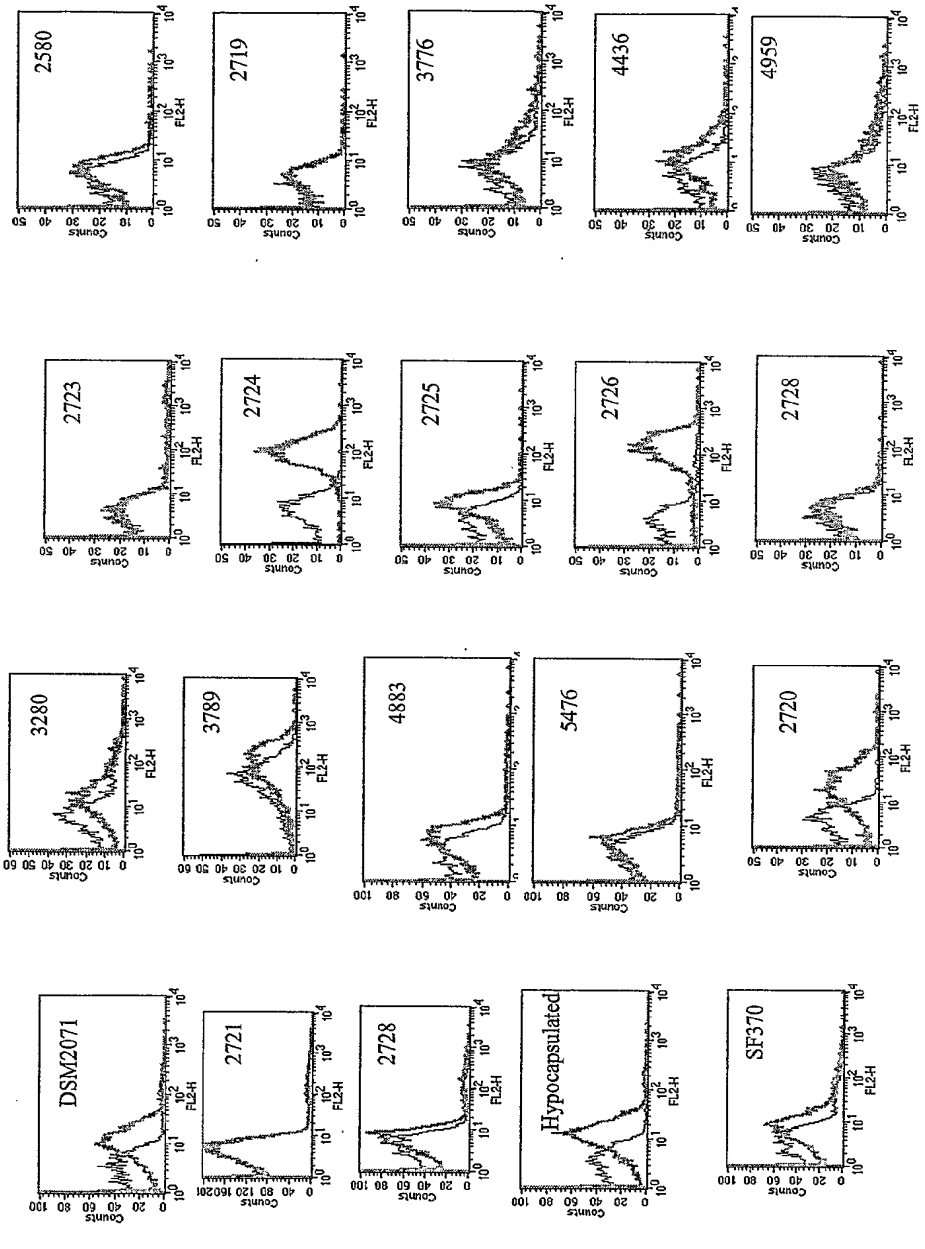
FIG. 10A
40aNH

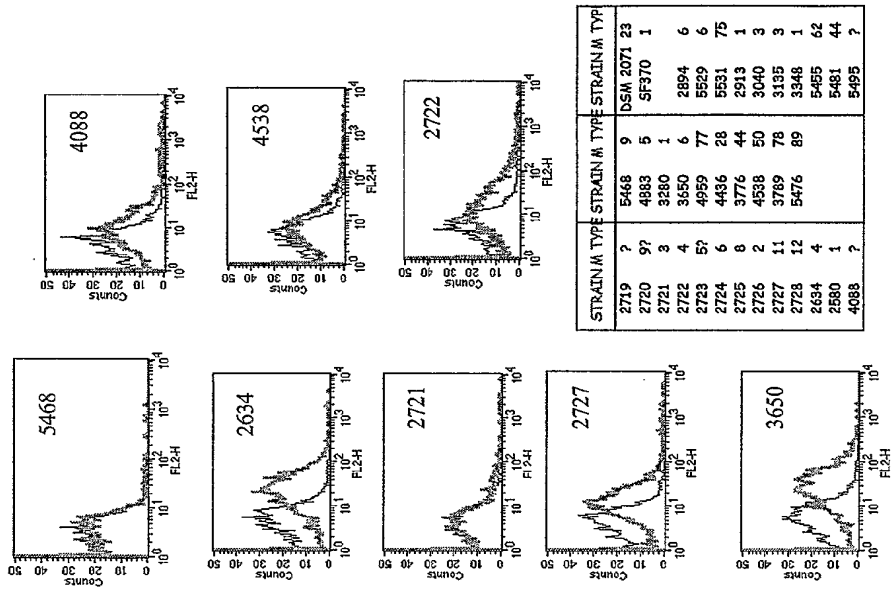


STRAIN #	TYPE	STRAIN #	TYPE	STRAIN #	TYP
2719	?	5468	9	DSM 2071	23
2720	??	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	??	4969	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5455	62
2580	1			5481	44
4088	?			5495	?

FIG. 10B
40aNH

FIG. 11A
40aRRNH



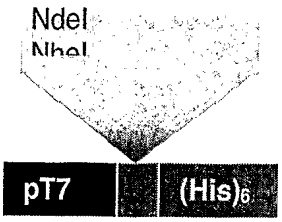


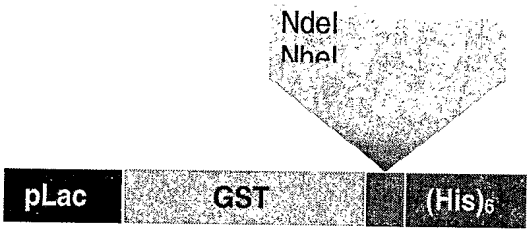
STRAIN M TYPE		STRAIN M TYPE		STRAIN M TYPE	
2719	?	5468	9	DSM 2071	23
2720	9?	4883	5	SF370	1
2721	3	3280	1		
2722	4	3650	6	2894	6
2723	5?	4959	77	5529	6
2724	6	4436	28	5531	75
2725	8	3776	44	2913	1
2726	2	4538	50	3040	3
2727	11	3789	78	3135	3
2728	12	5476	89	3348	1
2634	4			5455	62
2580	1			5481	44
4088	?			5495	?

FIG. 11B, 40aRRNH

FIG. 12A

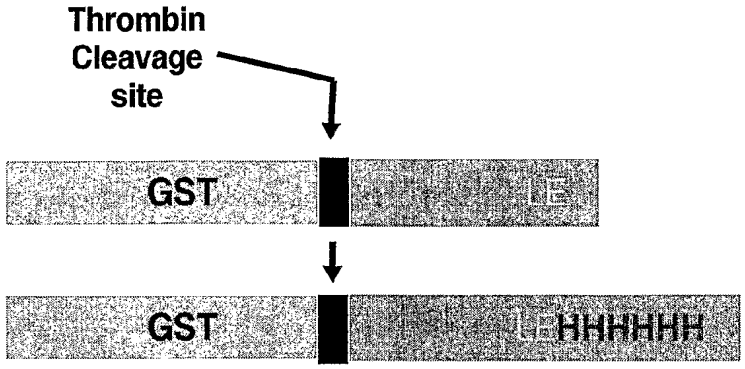
pET-21b+





pGEX-NNH

pET-21 b+



pGEX

FIG. 12B

FIG. 13. Mouse Model

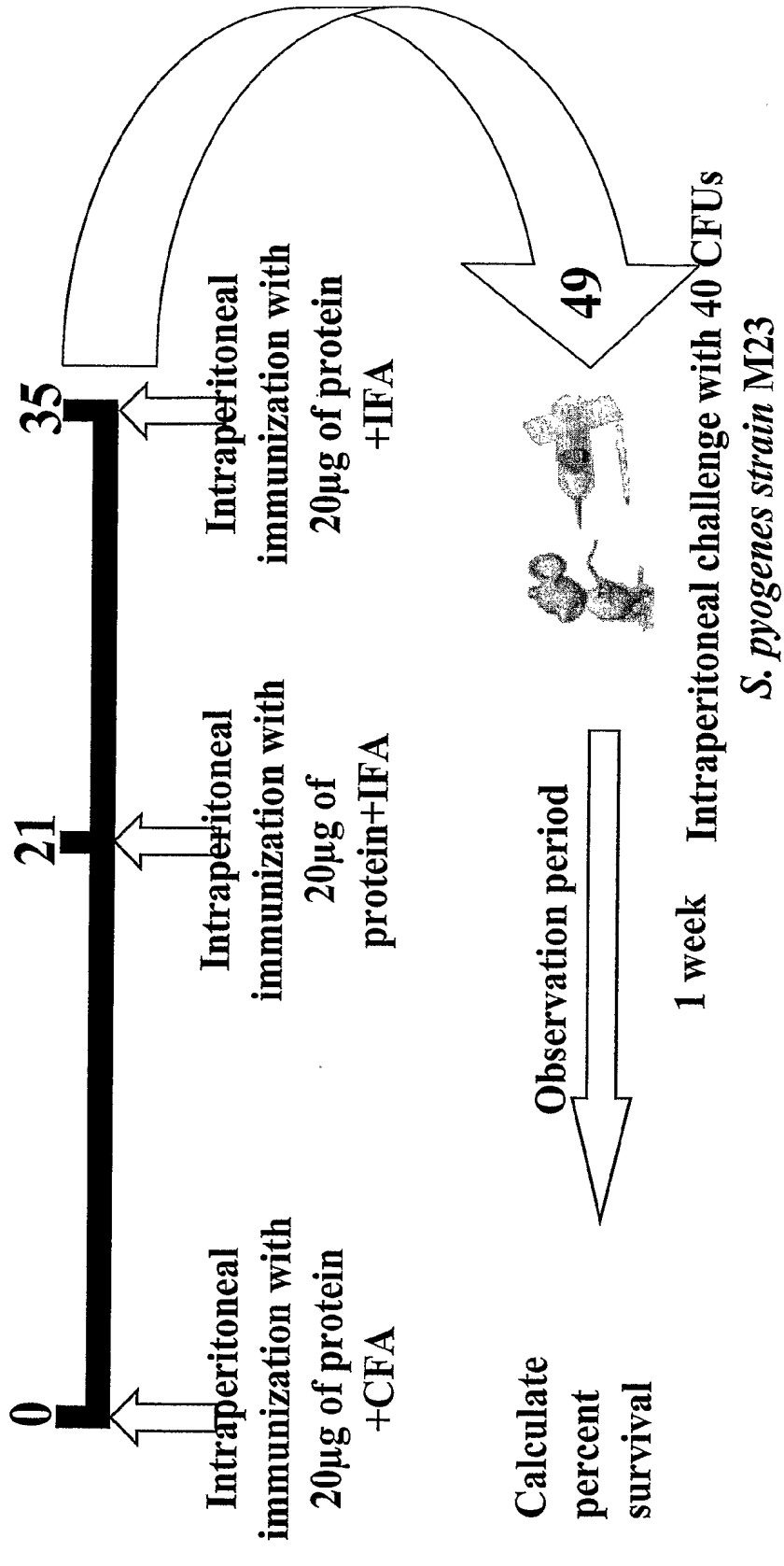


FIG. 14. Mouse Model Results

Antigen	Survival (%)	p-Value
GAS 40	51	< 0.0001
GAS 253	25	0.008
GAS 366	21	0.046
GAS 117	21	0.056
GAS 504	22	0.09
M homolog	99	
Negative control	12	

FIG. 15 Structure of GAS40

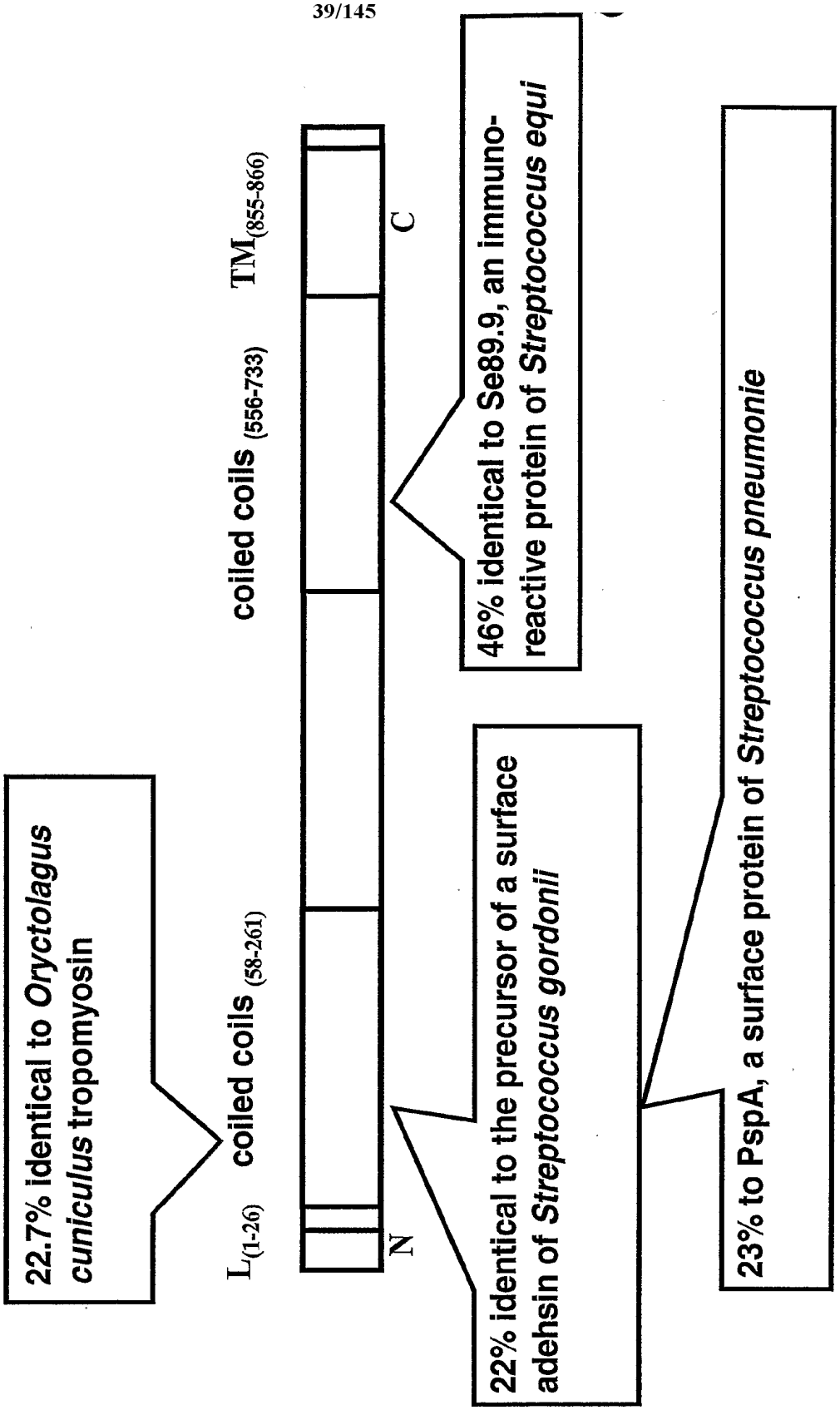
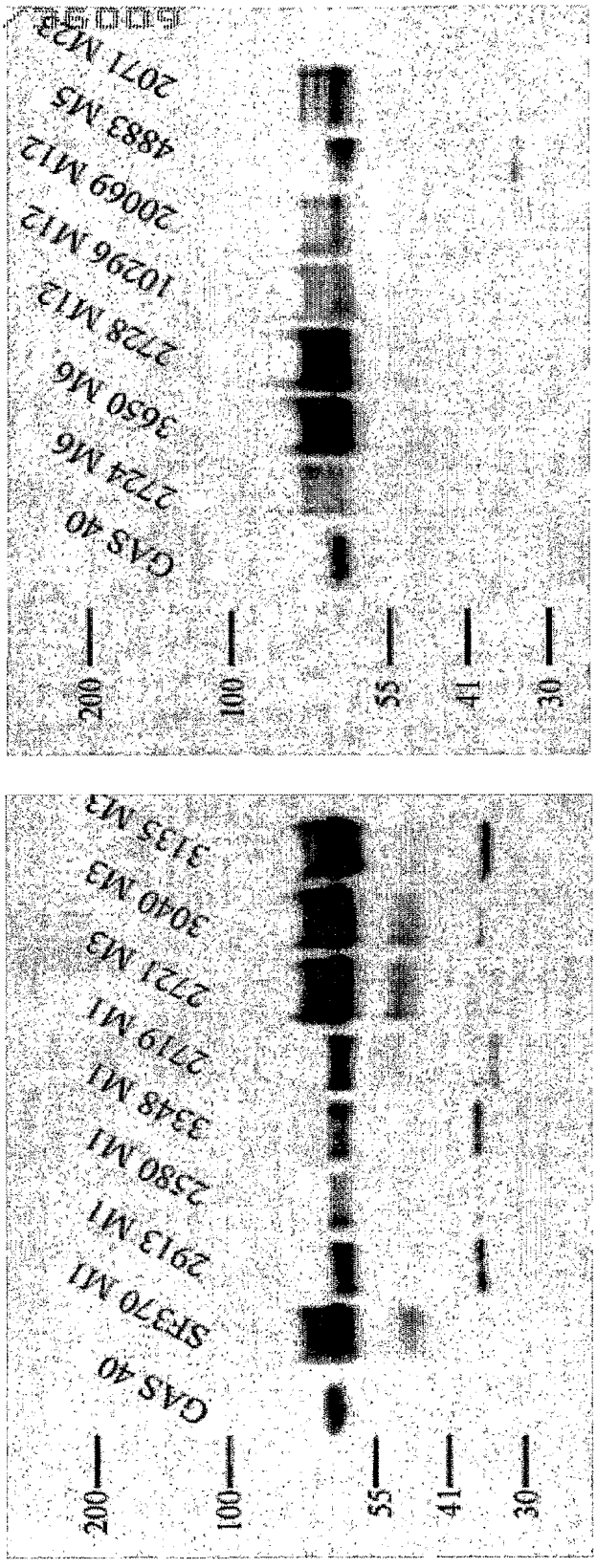


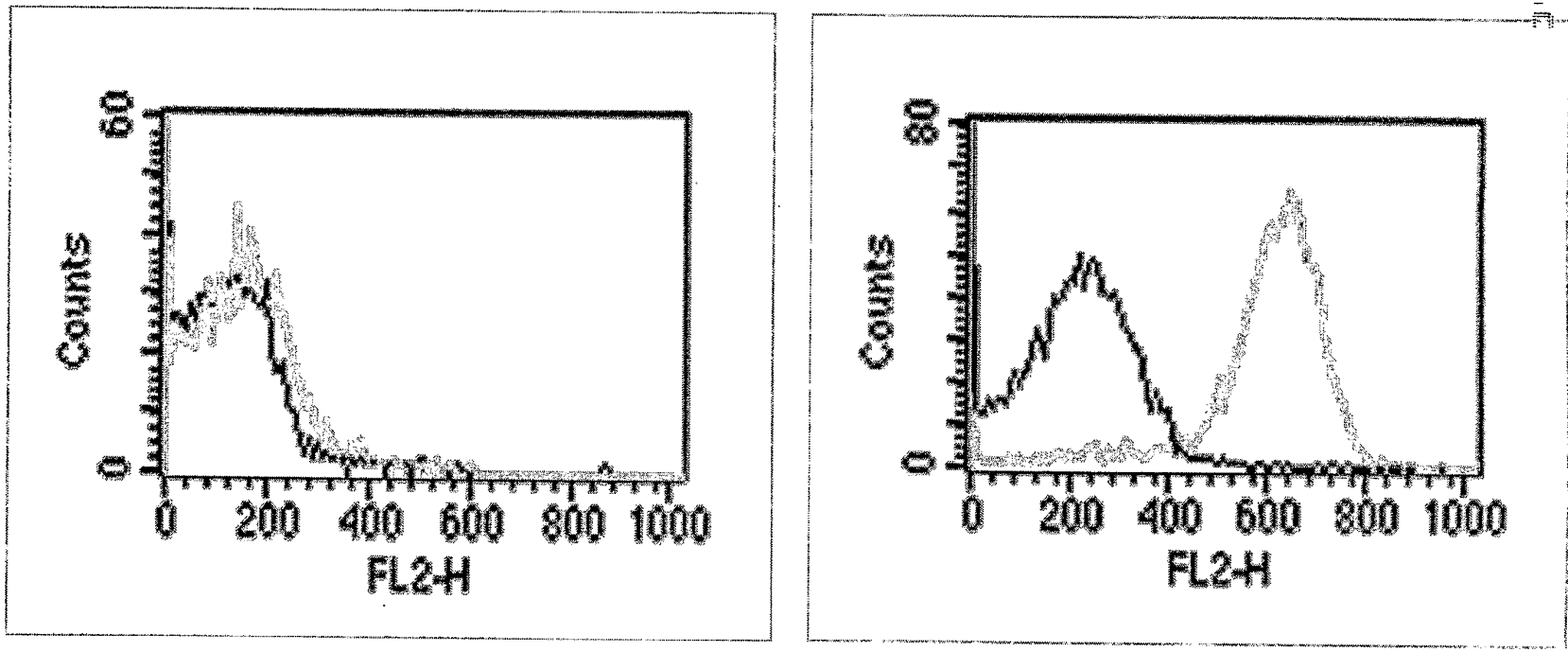
FIG. 16 Expression of GAS40 in different GAS serotypes



Anti-GAS 40

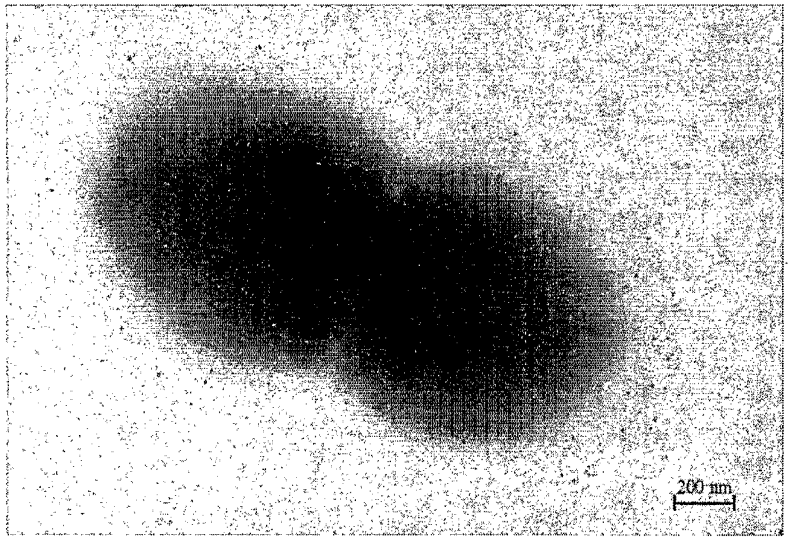
FIG. 17 Surface expression of GAS40

26 stocks of GAS used



12% **Anti-GAS 40** **88%**

FIG. 18 Distribution of GAS40 on the bacterial surface



preimmune

immune

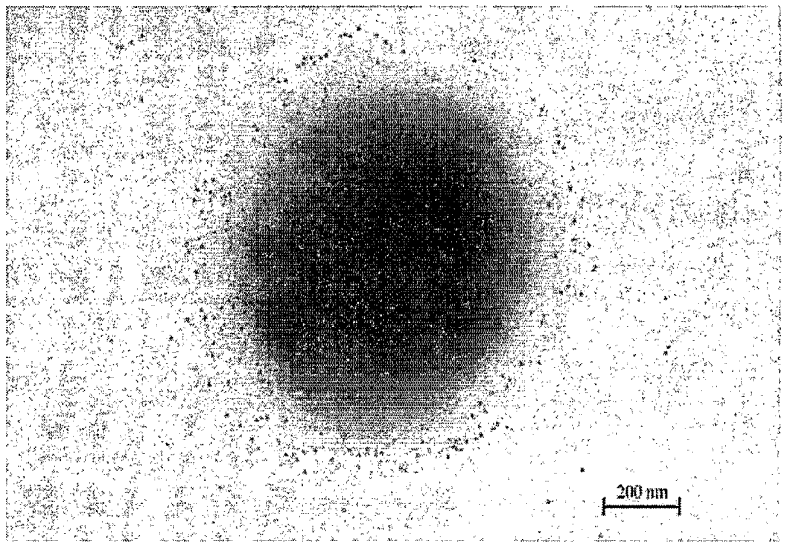


FIG. 19 Bacteriocidal properties of anti-GAS40 antibodies

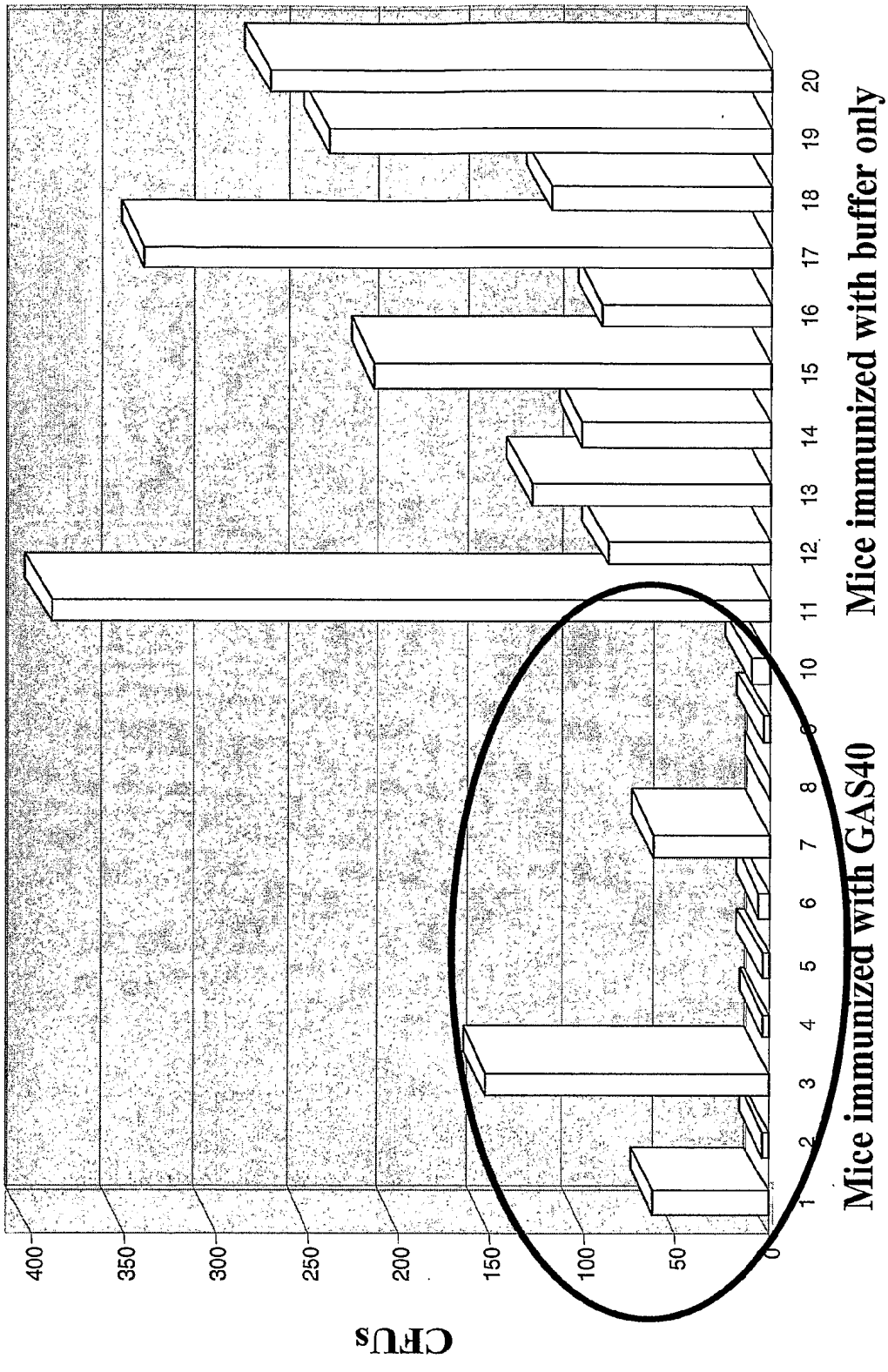


FIG. 20 Opsonization properties of anti-GAS 40 antibodies

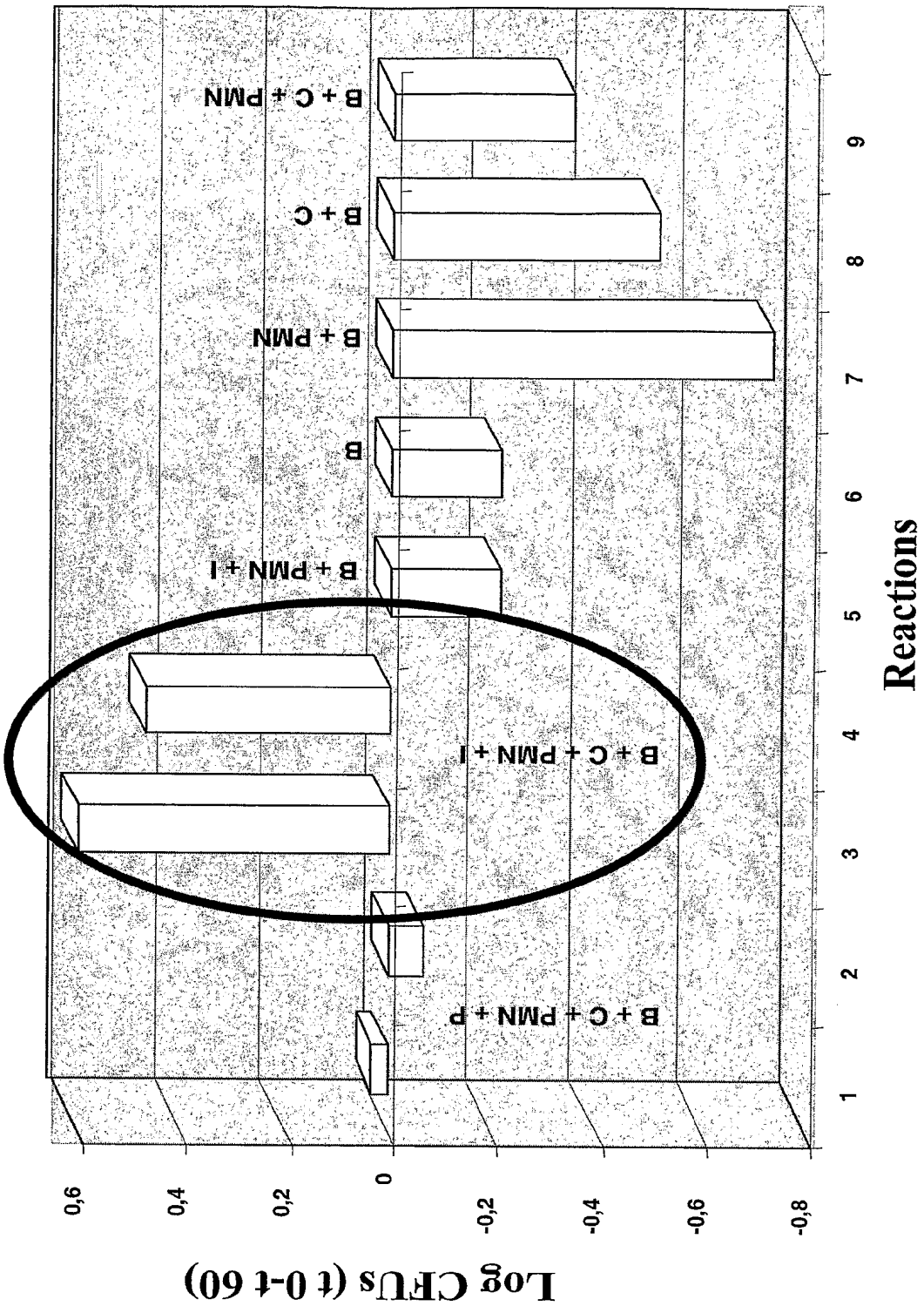


FIG. 21 GAS40 Domains

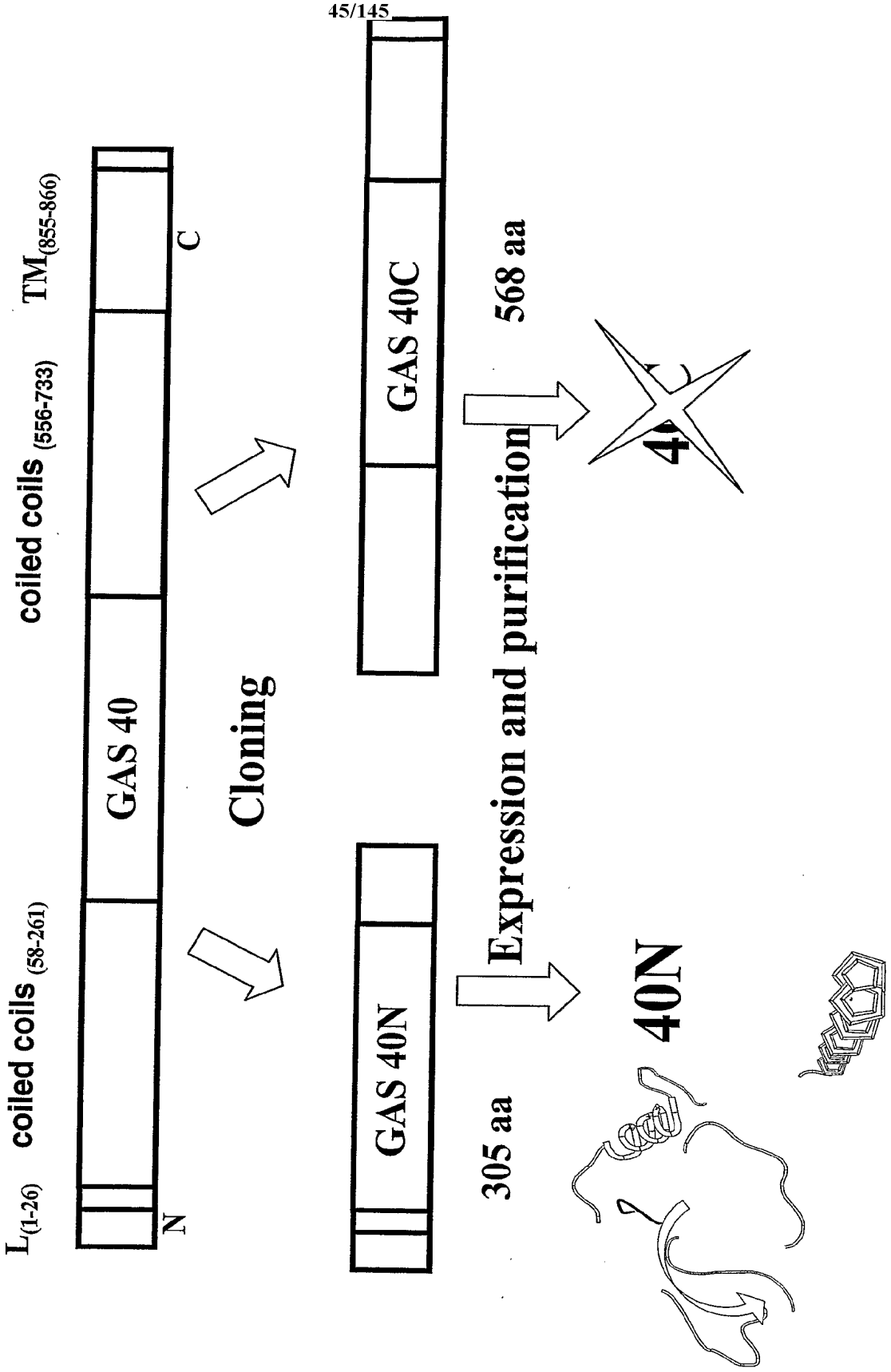
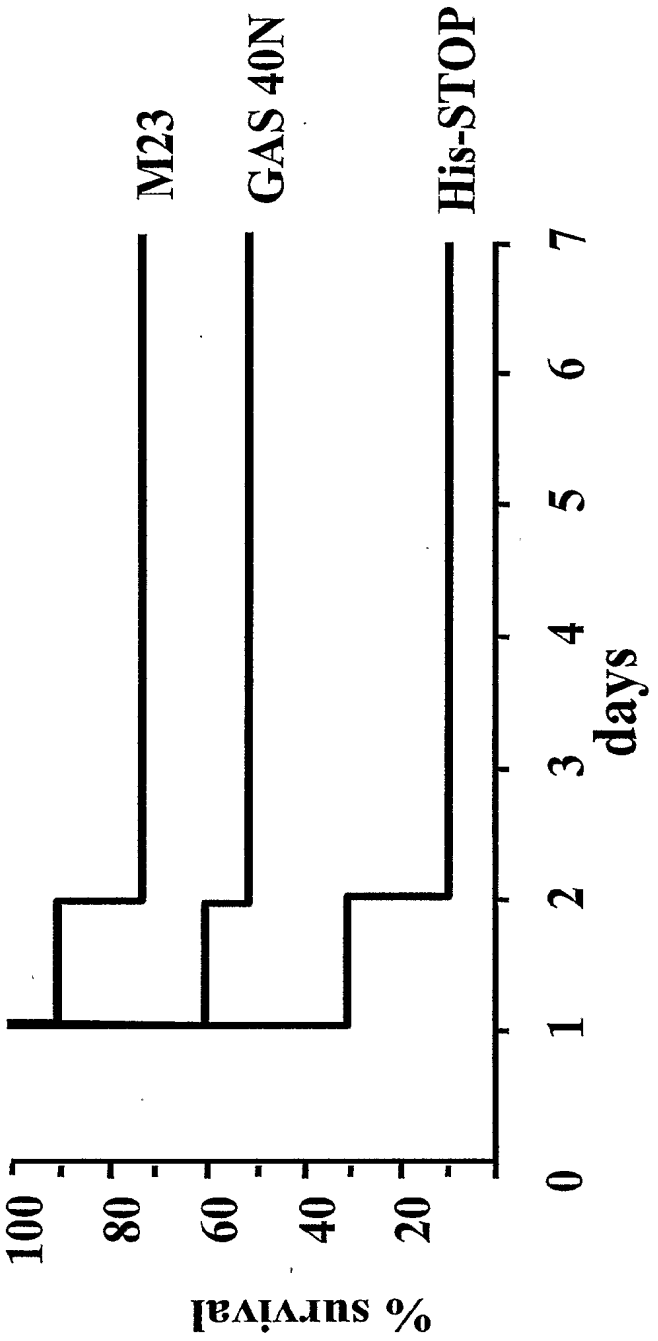
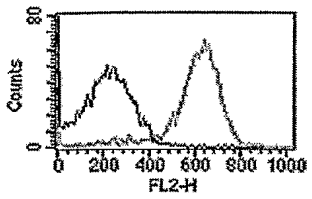


FIG. 22 GAS 40N



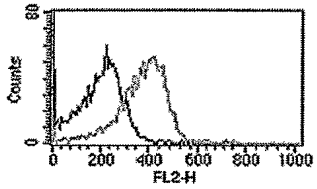
a)

STRAIN	SEROTYPE	ΔMean
SF370	1	323
3348	1	271
2726	2	400
2634	4	308
2724	6	277
2894	6	338
3650	6	322
2725	8	252
2720	9	452
2728	12	384
DSM2071	23	351
4436	28	294
5529	6	220



b)

STRAIN	SEROTYPE	ΔMean
2580	1	164
2913	1	89
3280	1	110
3135	3	132
2723	5	163
2727	11	89
3040	3	134
5476	89	185
5468	9	91
4883	5	83



c)

STRAIN	SEROTYPE	ΔMean
2719	1	8
2721	3	8
2722	4	27

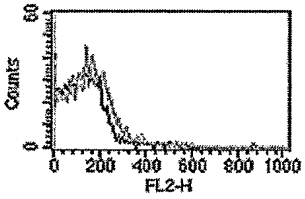
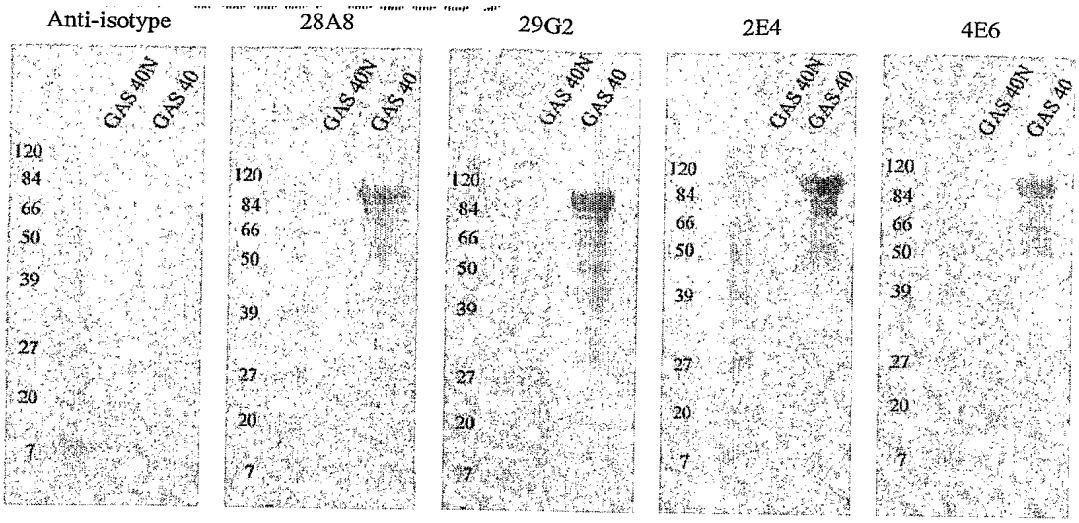


FIG. 23


Anti-isotype
28A8
29G2
2E4
4E6
GAS 40N
GAS 40
120
84
66
50
39
27
20
7

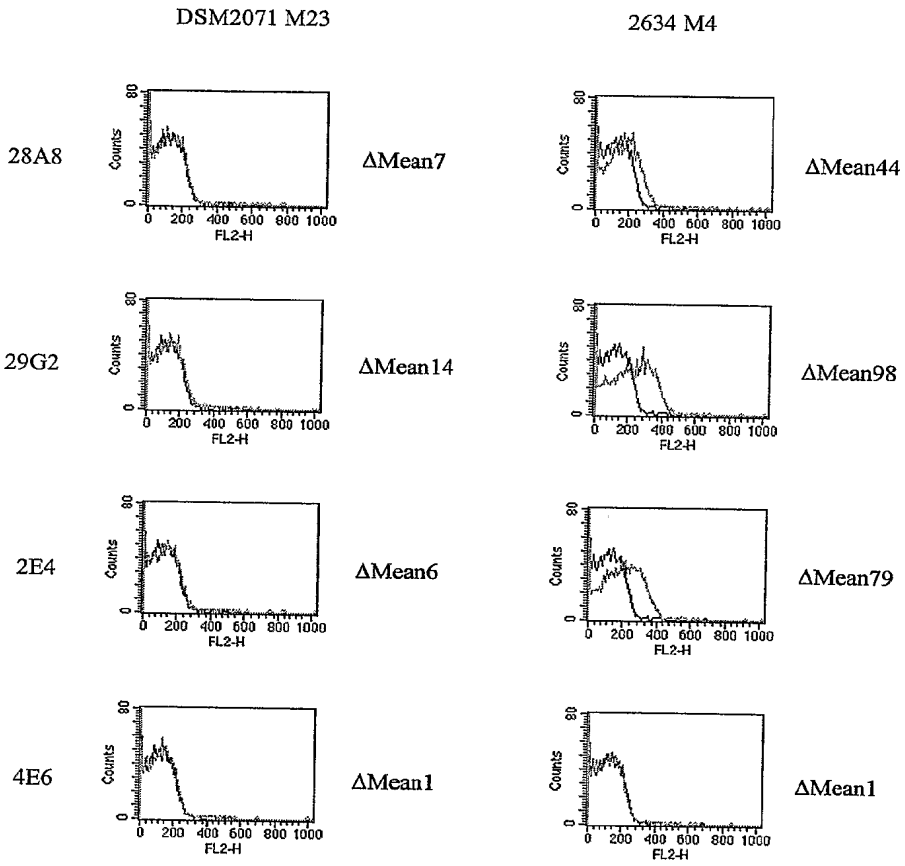

DSM2071 M23
2634 M4
28A8
ΔMean7
ΔMean44
29G2
ΔMean14
ΔMean98
2E4
ΔMean6
ΔMean79
4E6
ΔMean1
ΔMean1
Counts
FL2-H
0 200 400 600 800 1000

FIG. 24

FIG. 25A

MRRAENNKHSRYSIRKLSVGVTSIAIASLFLGKVAYAVDGIPPISLTQKTTATTSENWHHIDKDGLIPLGISLEAAKEEFKKEVEESRLSEA
EAAKEEFKKEVEES LSEA
EA

QKETYKQKIKTAPDKDKLLFTYHSEYMTAVKDLPASTESTTQPVEAPVQETQASASDSMVTGDSTSVTTDSPEETPSSESPVAPALSEAPAQ
QKETYKQKIKTAPDKDK ESTTQPVEAPVQETQ SVTTDSPEETPSSESPVAPAL
QKETYKQKIK STTQPVEAPVQETQ TDSPEETPSSESPVAPAL
KETYKQKIKTAPDKDKLLF DSPEETPSSESPVAPALS
KETYKQK DSPEETPSSESPVAP
EETPSSESPVAPALSE
LSEAPAQ
SEAPAQ
EAPAQ

PAESEEPSVAASSEETPSPSTPAAPETPEEPAAPSPSPESEEPSVAAPSEETPSPETPEEPAAPSQPAESEESSVAATTSPSPSTPAESETQ
PAESEEPSVA
PAESEEPSVA
PAESEEPSVA

TPPAVTKDSDKPSSAAEKPAASSLVSEQTVQQPTSKRSSDKKEEQEQSYSPNRSLSRQVRAHESGKYLPSTGEKAQPLFIATMTLMSLFGSL

LVTKRQKETKK

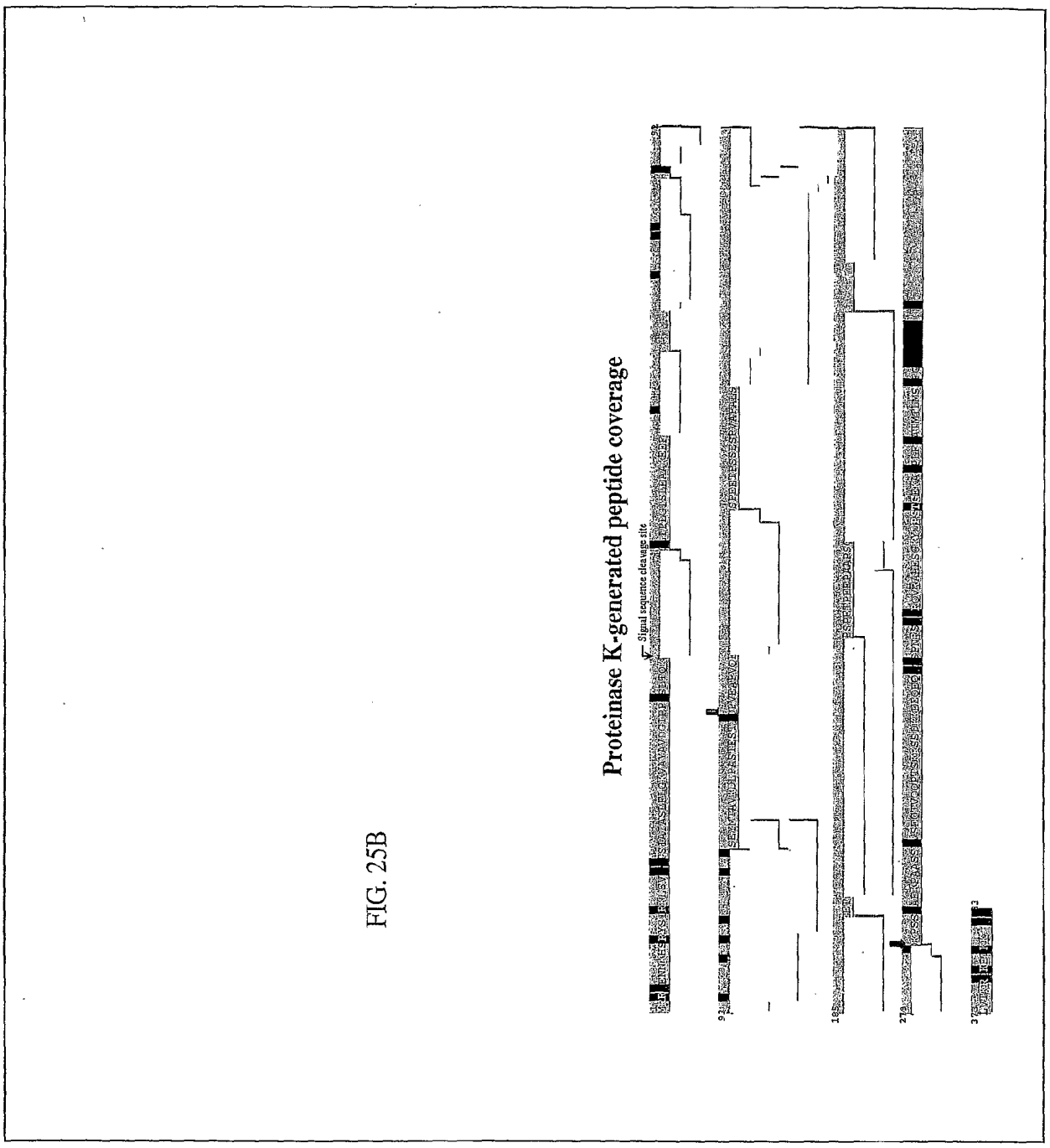

Proteinase K-generated peptide coverage
Signal sequence cleavage site

FIG. 25B

FIG. 26A

MRRAENNKHSRYSIRKLSVGVTSIAIASLFLGKVAYAVDGIPPISLTQKTTATTSENWHHIDKDGLIPLGISLEAAKEEFKKEVEESRLSEA

VDGIPPISLTQK TATTSENWHHIDK LSEA

DGLIPLGISLEAAK

EEFKKEVEESR

QKETYKQKIKTAPDKDKLLFTYHSEYMTAVKDLPASTESTTQPVEAPVQETQASASDSMVTGDSTSVTTDSPEETPSSESPVAPALSEAPAQ

QKETYKQK LLFTYHSEYMTAVK

IKTAPDKDKLLFTYHSEYMTAVK

PAESEEPSVAASSEETPSPSTPAAPETPEEPAAPSPSPESEEPSVAAPSEETPSPETPEEPAAPSQPAESEESSVAATTSPSPSTPAESETQ

TPPAVTKDSDKPSSAAEKPAASSLVSEQTVQQPTSKRSSDKKEEQEQSYSPNRSLSRQVRAHESGKY**LPSTG**EKAQPLFIATMTLMSLFGSL

DSDKPSSAAEKPAASSLVSEQTVQQPTSK

DSDKPSSAAEKPAASSLVSEQTVQQPTSKR

SSDKKEEQEQSYSPNR

EEQEQSYSPNR

LVTKRQKETKK

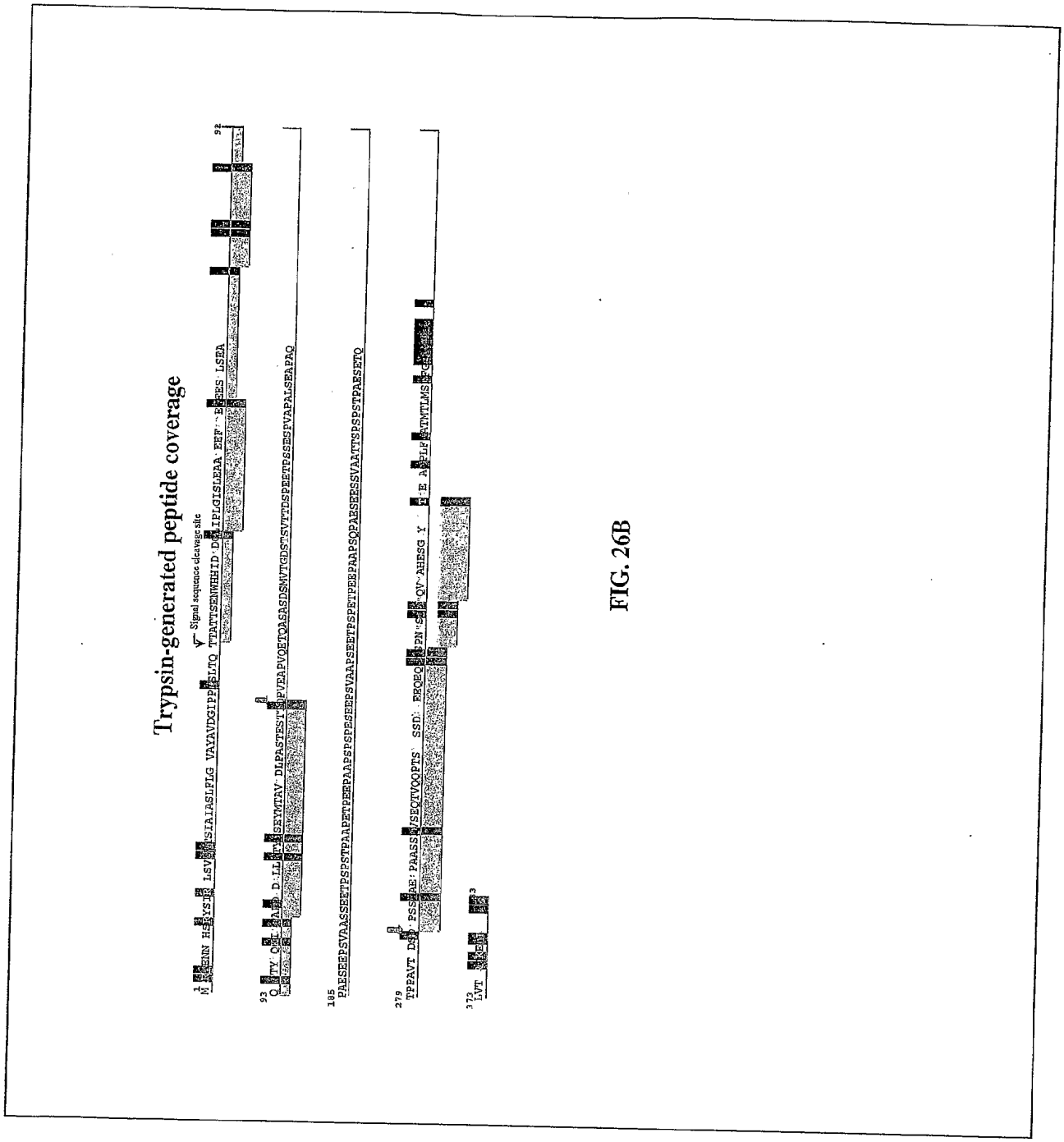

Trypsin-generated peptide coverage
Signal sequence cleavage site

FIG. 26B

FIG. 27

LPXTG proteins, 17 predicted

Identified	FACS positive	FACS negative	Not Cloned
12	11		1

Membrane proteins, 506 predicted

Identified	FACS positive	FACS negative	Not Cloned
37	17		15

Lipoproteins, 28 predicted

Identified	FACS positive	FACS negative	Not Cloned
11	9		-

Extracellular, 67 predicted

Identified	FACS positive	FACS negative	Not Cloned
8	6		1

Cytoplasmic

Identified	FACS positive	FACS negative	Not Cloned
8	-		8

GAS	SEQ	FACS response

Features: LPXTG

PSORT prediction

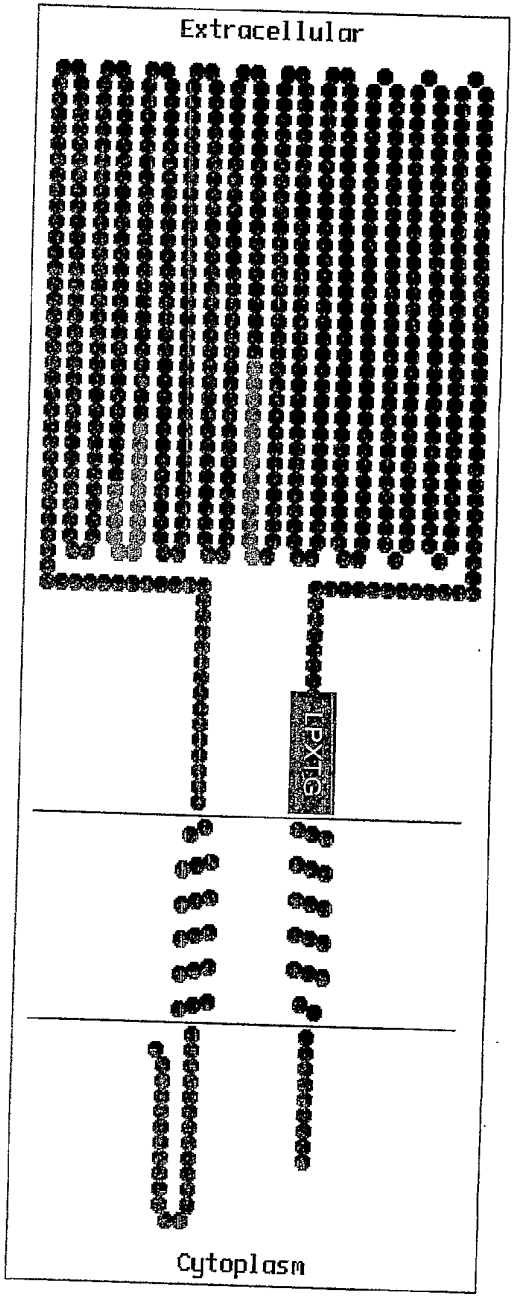
Signal Score (-7.5): 4.2 Possible cleavage site: 47
>>> Seems to have no N-terminal signal seq.

count: 2 value: -8.17 threshold: 0.0
INTEGRAL Likelihood = -8.17 Transmembrane 26 - 42 (24 - 54)
INTEGRAL Likelihood = -5.63 Transmembrane 732 - 748 (729 - 751)
PERIPHERAL Likelihood = 7.85 modified
ALOM score: 2.13

Rule: cytoplasmic membrane protein

FIG. 28

GAS15, SPY NT01SP0102, FACS RESPONSE POSITIVE



GAS	SPY	FACS response
		Positive

Features: LPXTG

PSORT prediction

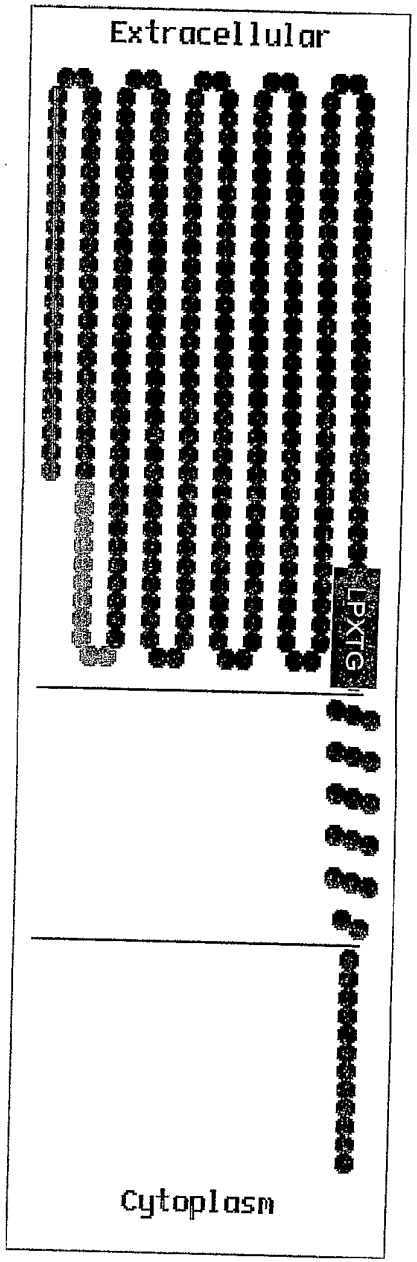
Signal Score (-7.5): 3.15 Possible cleavage site: 17
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -3.93 threshold: 0.0
INTEGRAL Likelihood = -3.93 Transmembrane 312 - 328 (311 - 337)
PERIPHERAL Likelihood = 9.12 modified
ALOM score: 1.29

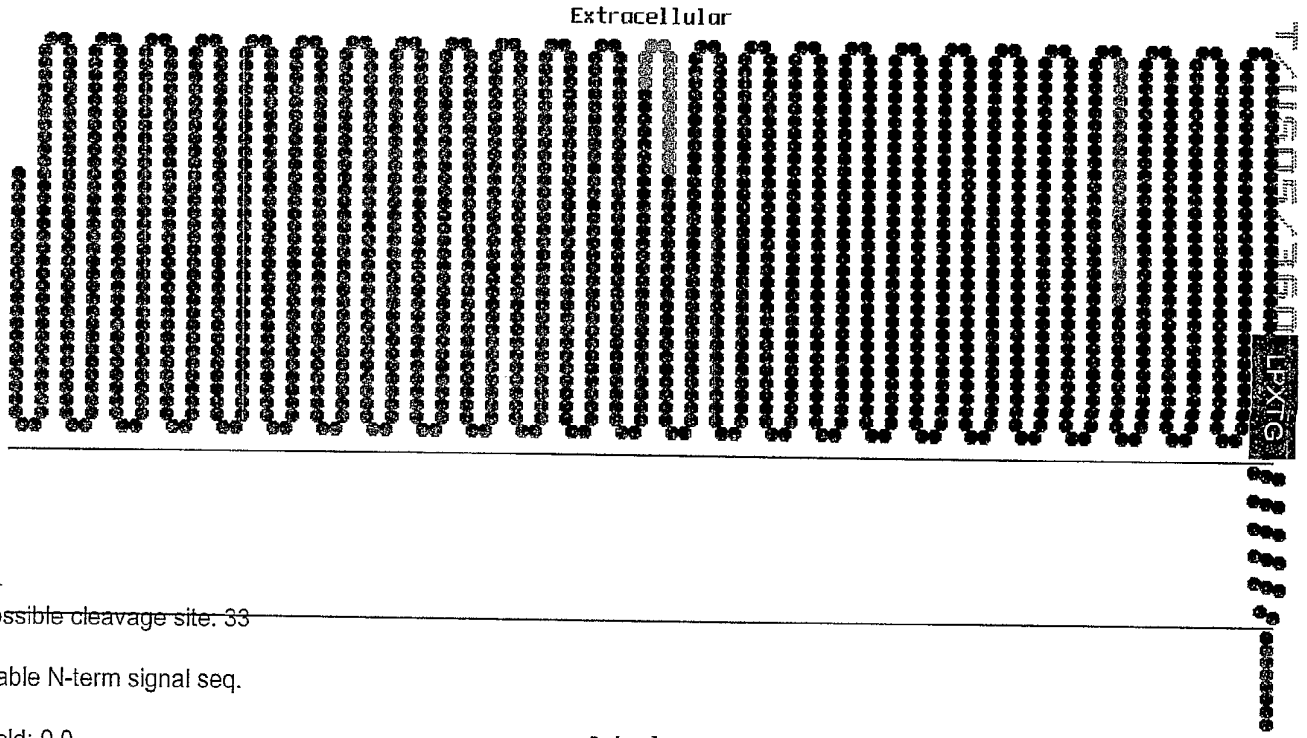
Rule: cytoplasmic membrane protein

FIG. 29

GAS16, SPY 0128, FACS RESPONSE POSITIVE



GAS	SPY	FACS response
		Positive



Features: LPXTG

PSORT prediction

Signal Score (-7.5): -0.35 Possible cleavage site: 33

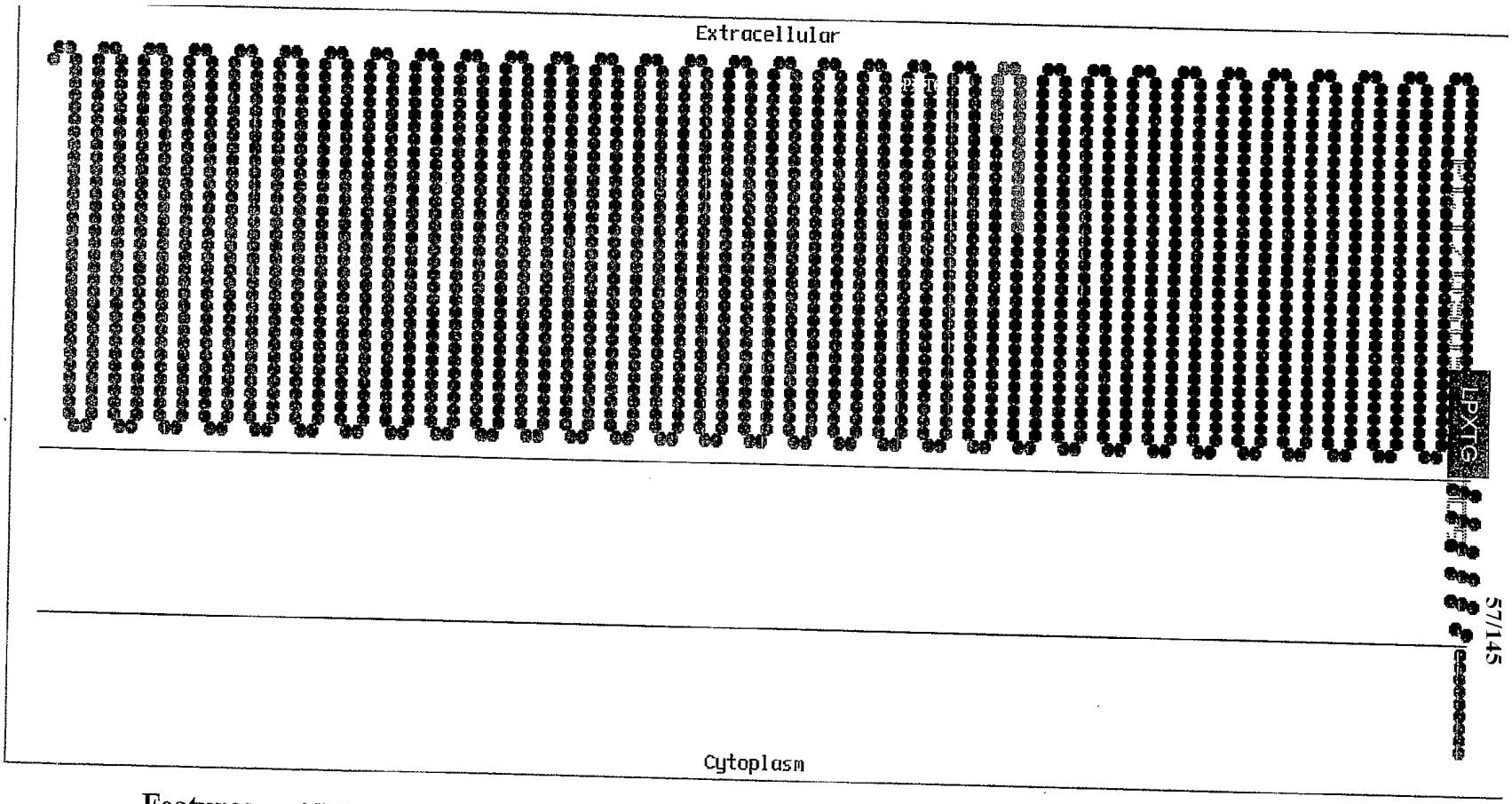
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -4.46 threshold: 0.0
INTEGRAL Likelihood = -4.46 Transmembrane 1623 -1639 (1621 -1641)
PERIPHERAL Likelihood = 1.16 modified ALOM score: 1.39

Rule: cytoplasmic membrane protein

FIG. 30

GAS57, SPY 0416, FACS RESPONSE POSITIVE



Features: LPXTG

PSORT prediction

Signal Score (-7.5): -2.32 Possible cleavage site: 38
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 6.74 threshold: 0.0

PERIPHERAL Likelihood = 6.74 modified ALOM score: -1.85

FIG. 31
GAS68, SPY 0737, FACS RESPONSE POSITIVE

GAS	SPY	FACS response
		Positive

Features: LPXTG

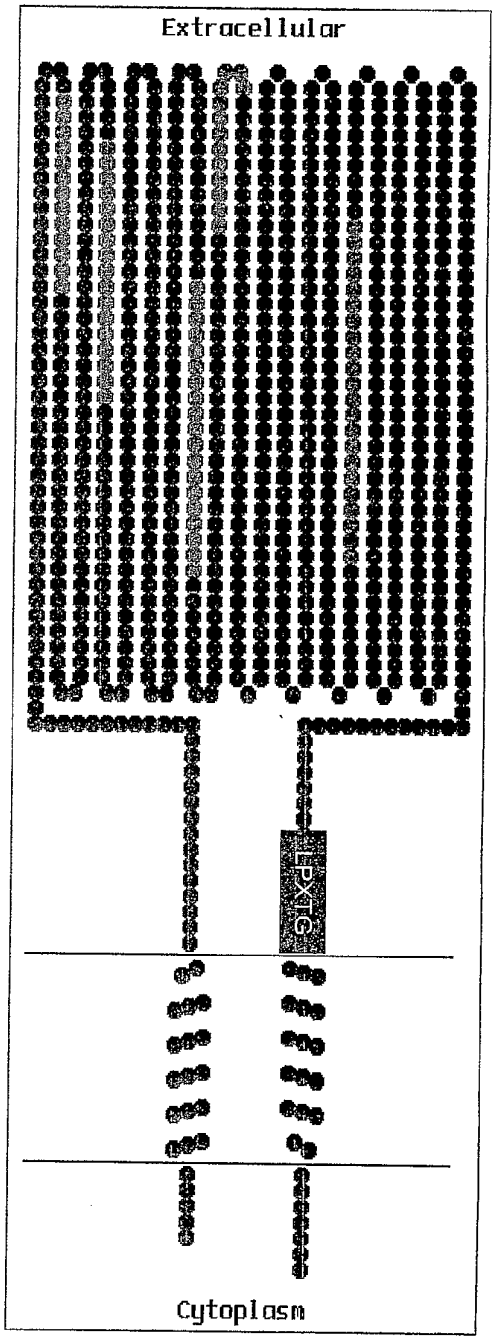
PSORT prediction

Signal Score (-7.5): -4.11 Possible cleavage site: 22
>>> Seems to have an uncleavable N-term signal seq

count: 2 value: -7.80 threshold: 0.0
INTEGRAL Likelihood = -7.80 Transmembrane 887 - 903 (882 - 906)
INTEGRAL Likelihood = -4.88 Transmembrane 6 - 22 (5 - 23)
PERIPHERAL Likelihood = 2.28 modified ALOM score: 2.06

Rule: cytoplasmic membrane protein

FIG. 32
GAS143, SPY 0747, FACS RESPONSE POSITIVE



GAS	SPY	FACS response
		Positive

Features: LPXTG

PSORT prediction

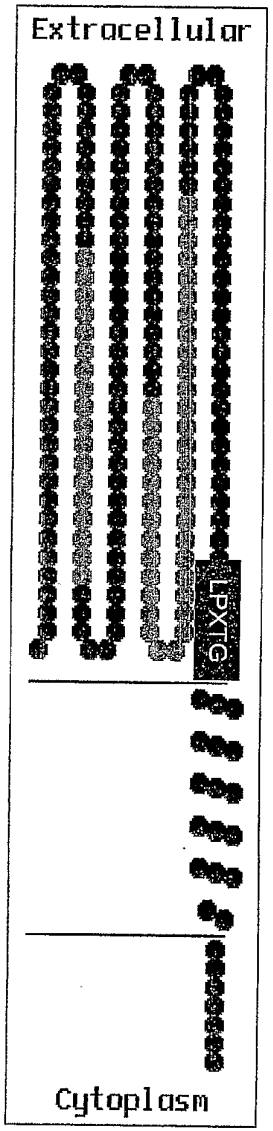
Signal Score (-7.5): -1.13 Possible cleavage site: 33

>>> Seems to have a cleavable N-term signal seq.

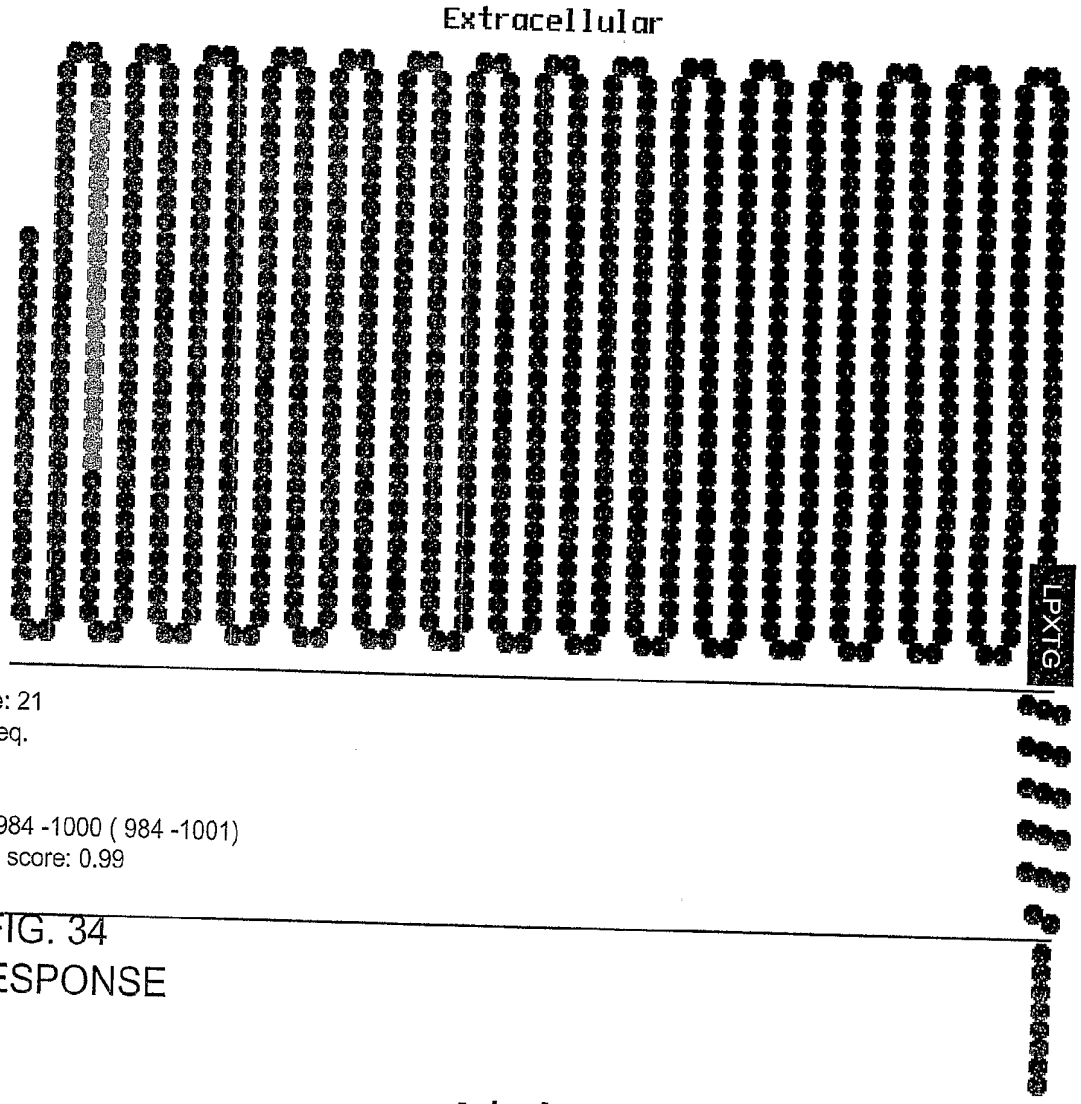
count: 1 value: -6.00 threshold: 0.0
INTEGRAL Likelihood = -6.00 Transmembrane 194 - 210 (192 - 214)
PERIPHERAL Likelihood = 8.33 modified ALOM score: 1.70

Rule: cytoplasmic membrane protein

FIG. 33
GAS166, SPY 1357, FACS RESPONSE POSTIIVE



ORF	name	FACS response
gas158	spy0843	Positive



Features: LPXTG

PSORT prediction

Signal Score (-7.5): -1.29 Possible cleavage site: 21
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -2.44 threshold: 0.0
INTEGRAL Likelihood = -2.44 Transmembrane 984 -1000 (984 -1001)
PERIPHERAL Likelihood = 4.29 modified ALOM score: 0.99

Rule: cytoplasmic membrane protein

FIG. 34

GAS158, SPY 0843, FACS RESPONSE POSITIVE

GAS	SPY	FACS response
gas171	spy1494	NOT CLONED

Features: LPXTG

PSORT prediction

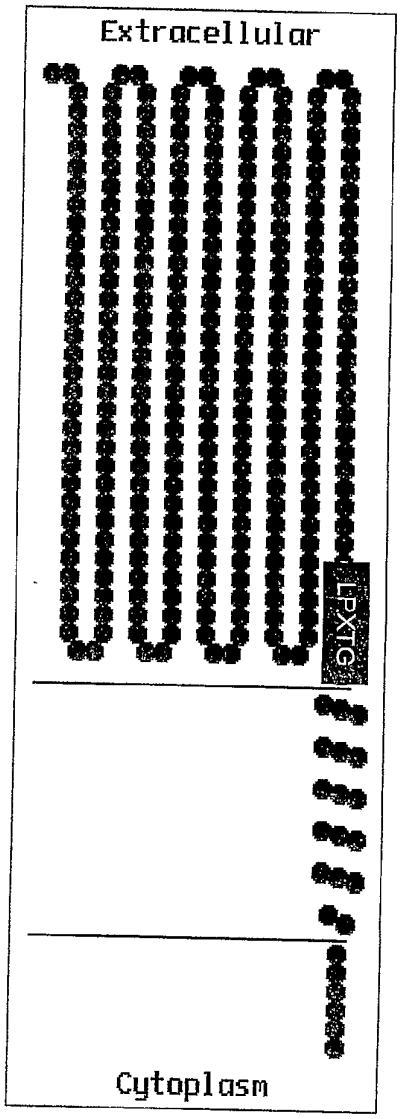
Signal Score (-7.5): -1.95 Possible cleavage site: 25

>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -4.04 threshold: 0.0
INTEGRAL Likelihood = -4.04 Transmembrane 291 - 307 (290 - 309)
PERIPHERAL Likelihood = 6.47 modified ALOM score: 1.31

Rule: cytoplasmic membrane protein

FIG. 35
GAS171, SPY 1494



ORF	SPY	FACS response
		Positive

Features: LPXTG

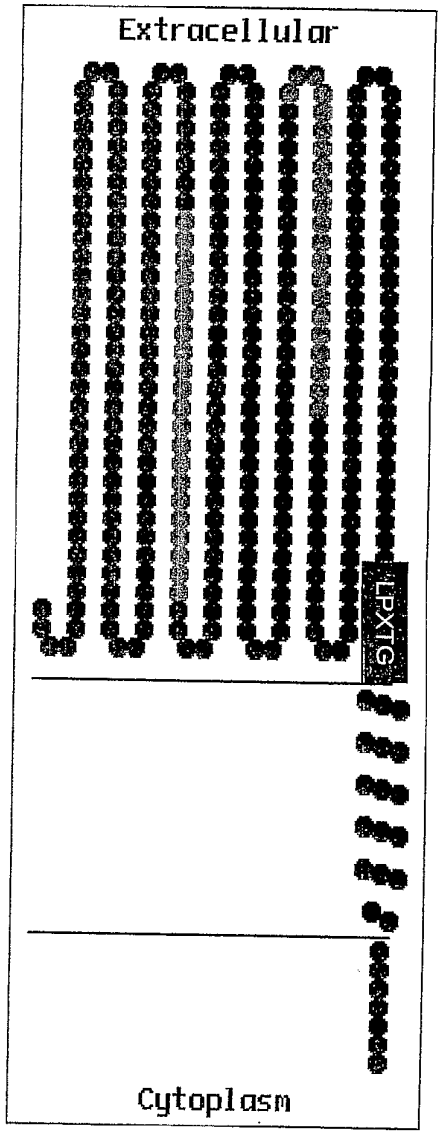
PSORT prediction

Signal Score (-7.5): 1.77 Possible cleavage site: 37
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -3.56 threshold: 0.0
INTEGRAL Likelihood = -3.56 Transmembrane 325 - 341 (323 - 343)
PERIPHERAL Likelihood = 16.29 modified ALOM score: 1.21

Rule: cytoplasmic membrane protein

FIG. 36
GAS188, SPY 1983, FACS RESPONSE POSITIVE



GAS	SPY	FACS response
		Positive

Features: LPXTG

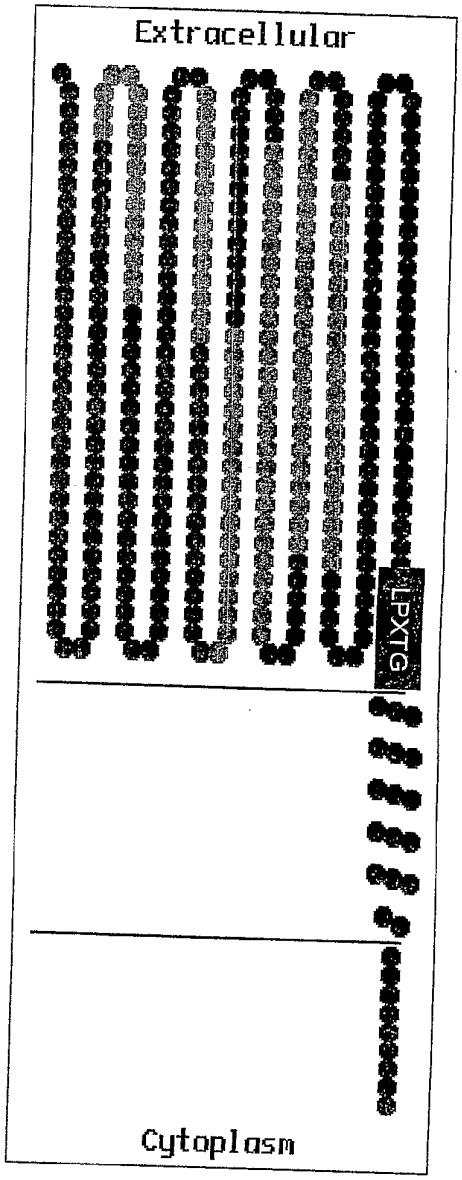
PSORT prediction

Signal Score (-7.5): -0.46 Possible cleavage site: 37
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -4.57 threshold: 0.0
INTEGRAL Likelihood = -4.57 Transmembrane 354 - 370 (353 - 371)
PERIPHERAL Likelihood = 10.18 modified ALOM score: 1.41

Rule: cytoplasmic membrane protein

FIG. 37
GAS190, SPY 2009, FACS RESPONSE POSITIVE



		FACS response
		Positive

Features: LPXTG

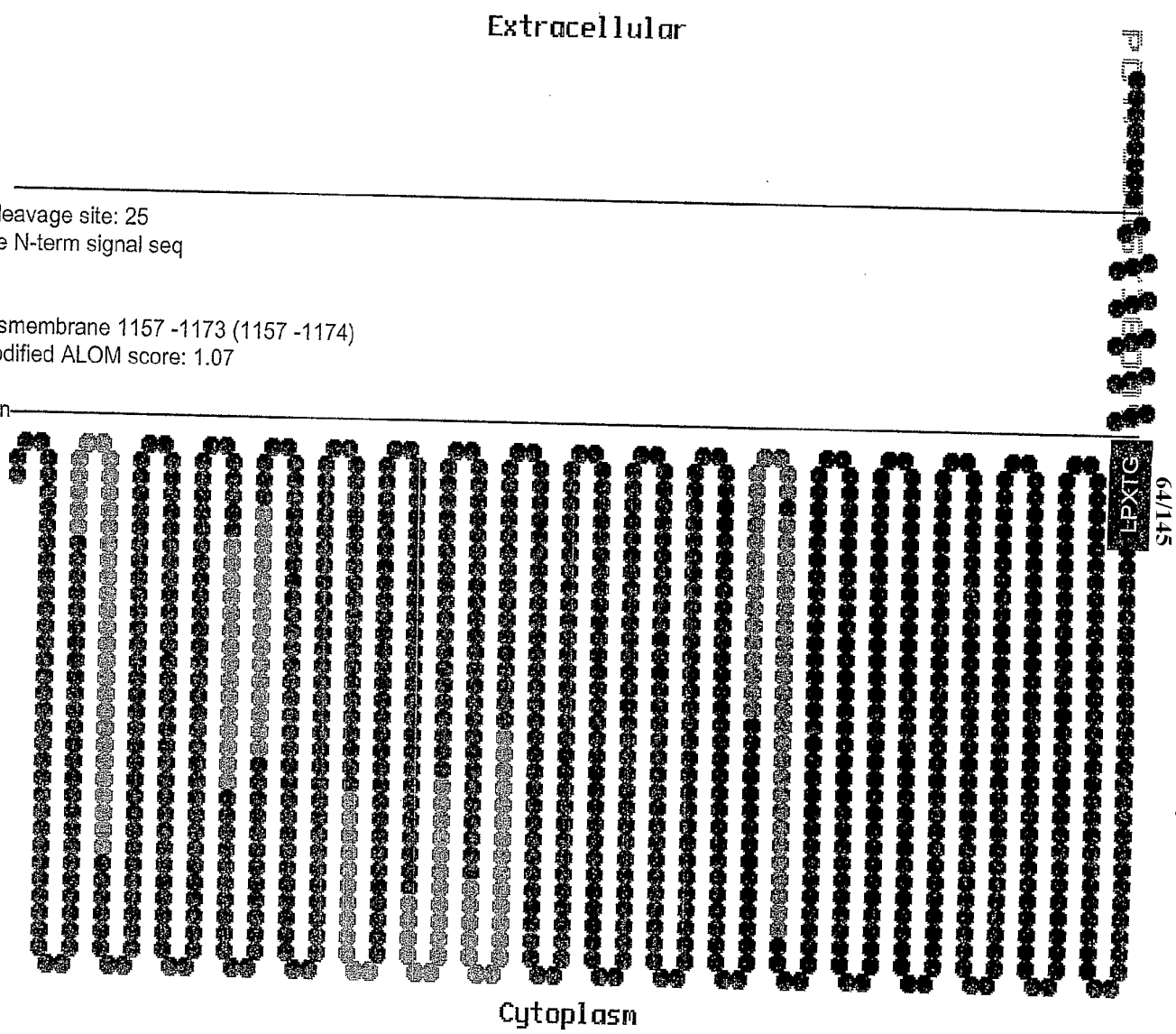
PSORT prediction

Signal Score (-7.5): -2.8 Possible cleavage site: 25
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -2.87 threshold: 0.0
INTEGRAL Likelihood = -2.87 Transmembrane 1157 -1173 (1157 -1174)
PERIPHERAL Likelihood = 1.01 modified ALOM score: 1.07

Rule: cytoplasmic membrane protein

FIG. 38
GAS191, SPY 2010,
FACS RESPONSE
POSITIVE



		FACS response
		Positive

Features: LPXTG

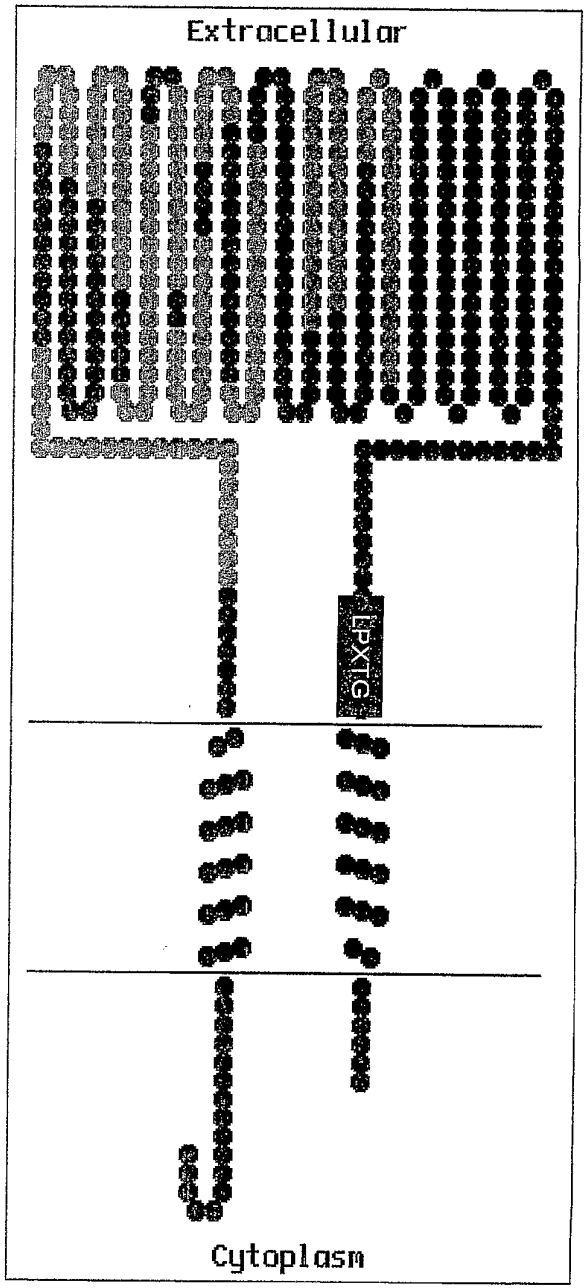
PSORT prediction

Signal Score (-7.5): -3.55 Possible cleavage site: 37
>>> Seems to have no N-terminal signal seq.

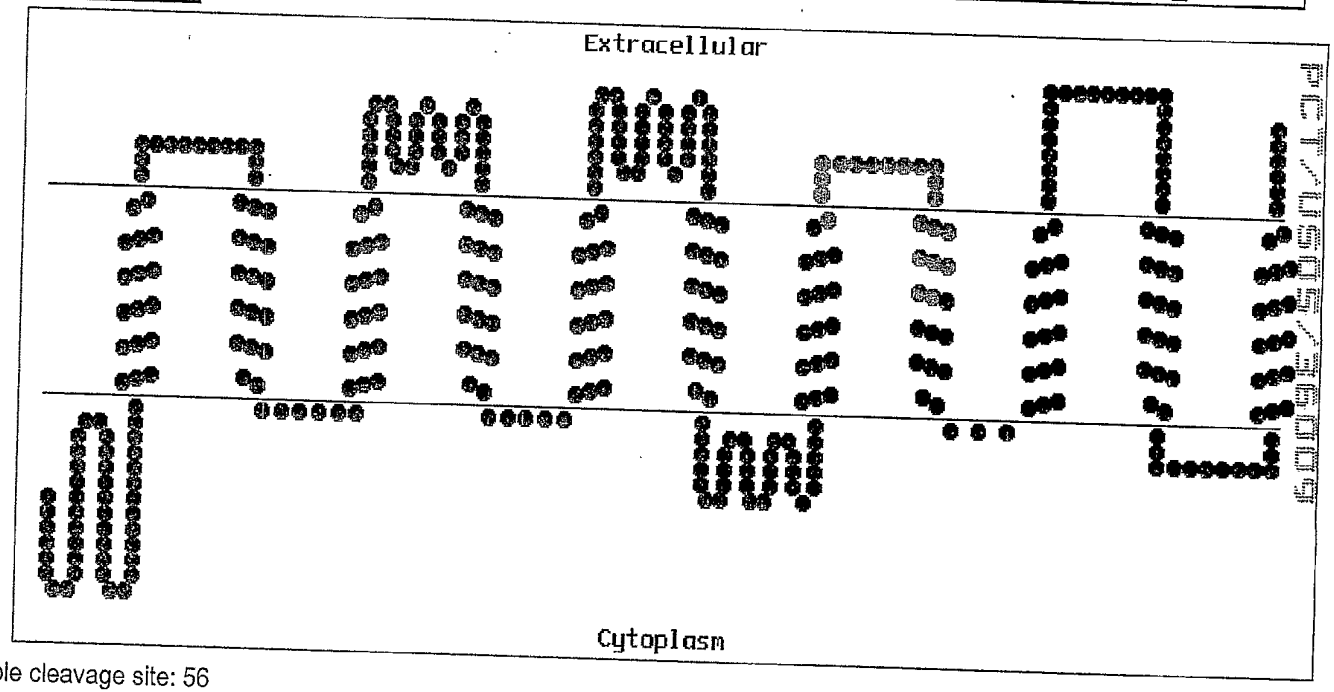
count: 2 value: -3.03 threshold: 0.0
INTEGRAL Likelihood = -3.03 Transmembrane 462 - 478 (460 - 479)
INTEGRAL Likelihood = -0.90 Transmembrane 18 - 34 (18 - 34)
PERIPHERAL Likelihood = 12.36 modified ALOM score: 1.11

Rule: cytoplasmic membrane protein

FIG. 39
GAS192, SPY 2019, FACS RESPONSE POSITIVE



		FACS response
		NOT CLONED



Features: Membrane

PSORT prediction

Signal Score (-7.5): -0.63 Possible cleavage site: 56
>>> Seems to have no N-terminal signal seq.

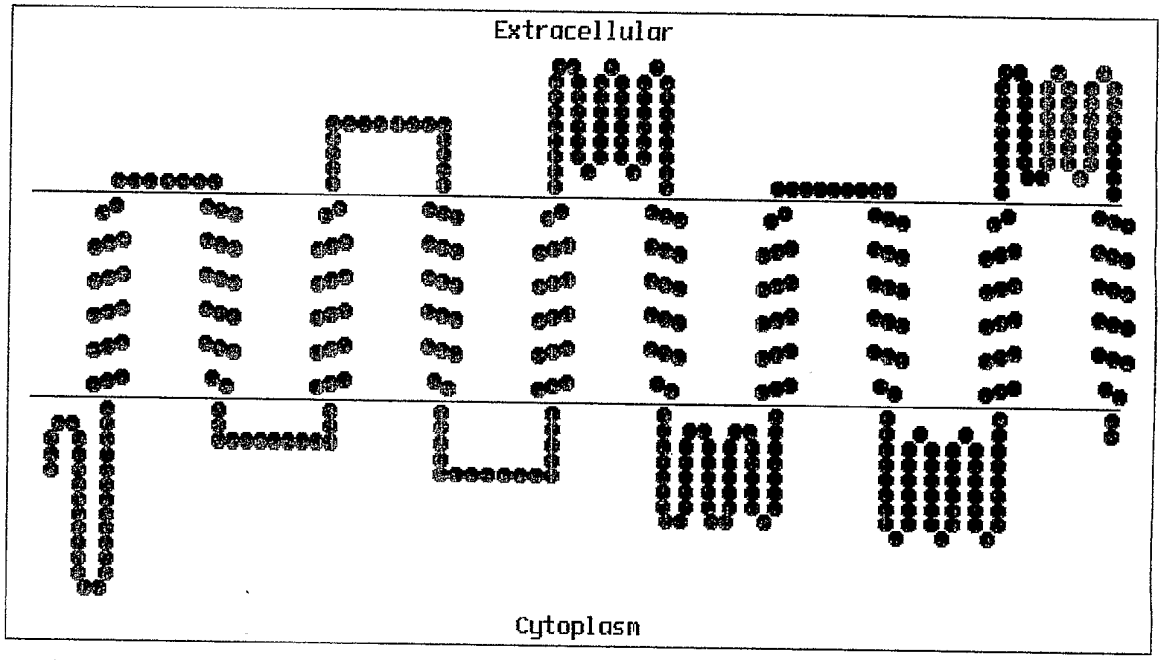
INTEGRAL Likelihood =-11.41 Transmembrane 166 - 182 (161 - 188)
INTEGRAL Likelihood = -7.75 Transmembrane 384 - 400 (376 - 403)
INTEGRAL Likelihood = -7.64 Transmembrane 266 - 282 (261 - 285)
INTEGRAL Likelihood = -4.25 Transmembrane 295 - 311 (291 - 313)
INTEGRAL Likelihood = -2.71 Transmembrane 98 - 114 (98 - 115)
INTEGRAL Likelihood = -2.23 Transmembrane 355 - 371 (355 - 374)
INTEGRAL Likelihood = -2.02 Transmembrane 218 - 234 (218 - 234)
INTEGRAL Likelihood = -1.91 Transmembrane 315 - 331 (315 - 331)
INTEGRAL Likelihood = -1.22 Transmembrane 75 - 91 (75 - 92)
INTEGRAL Likelihood = -0.75 Transmembrane 45 - 61 (45 - 63)
INTEGRAL Likelihood = -0.75 Transmembrane 144 - 160 (144 - 161)
PERIPHERAL Likelihood = 0.58 modified ALOM score: 2.78

Rule: cytoplasmic membrane protein
GAS650, SPY 2120

FIG. 40

11 TM

ORF	SPY	FACS response
gas558	spy1109	NOT CLONED



Features: Membrane

PSORT prediction

Signal Score (-7.5): -3.32 Possible cleavage site: 48
>>> Seems to have no N-terminal signal seq.

count: 10 value: -11.89 threshold: 0.0
INTEGRAL Likelihood =-11.89 Transmembrane 361 - 377 (350 - 383)
INTEGRAL Likelihood = -7.43 Transmembrane 84 - 100 (79 - 102)
INTEGRAL Likelihood = -6.16 Transmembrane 150 - 166 (137 - 171)
INTEGRAL Likelihood = -4.88 Transmembrane 30 - 46 (24 - 48)
INTEGRAL Likelihood = -4.35 Transmembrane 299 - 315 (297 - 316)
INTEGRAL Likelihood = -4.14 Transmembrane 117 - 133 (115 - 134)
INTEGRAL Likelihood = -3.19 Transmembrane 54 - 70 (51 - 75)
INTEGRAL Likelihood = -2.92 Transmembrane 425 - 441 (425 - 442)
INTEGRAL Likelihood = -2.81 Transmembrane 213 - 229 (209 - 232)
INTEGRAL Likelihood = -2.44 Transmembrane 273 - 289 (271 - 290)
PERIPHERAL Likelihood = 0.32 modified ALOM score: 2.88

Rule: cytoplasmic membrane protein FIG. 41

10 TM

ORF	spy>	FACS response
gas460	spy0572	NOT CLONED

Features: Membrane

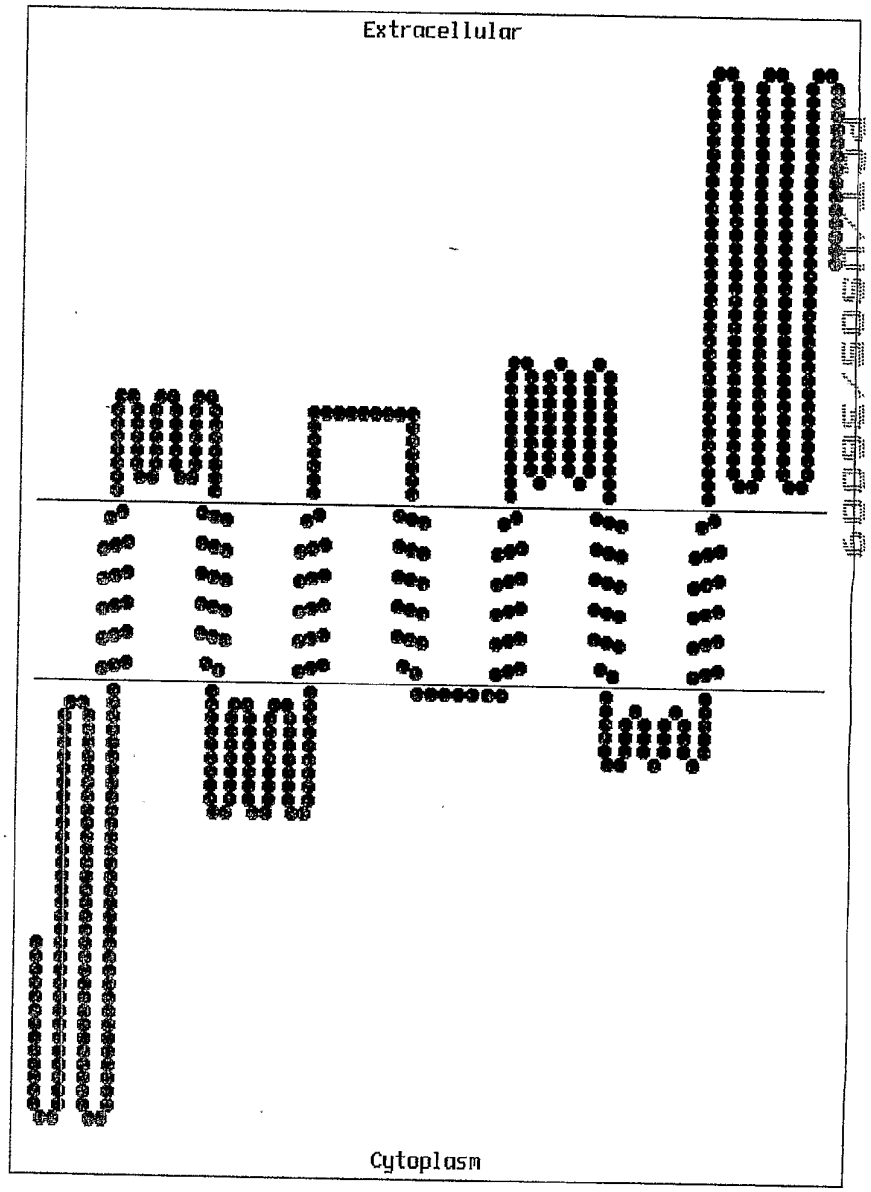
PSORT prediction

Signal Score (-7.5): -7.23 Possible cleavage site: 20
>>> Seems to have no N-terminal signal seq.

count: 7 value: -10.40 threshold: 0.0
INTEGRAL Likelihood =-10.40 Transmembrane 246 - 262 (240 - 271)
INTEGRAL Likelihood = -6.26 Transmembrane 284 - 300 (279 - 304)
INTEGRAL Likelihood = -4.14 Transmembrane 173 - 189 (172 - 194)
INTEGRAL Likelihood = -3.24 Transmembrane 112 - 128 (111 - 137)
INTEGRAL Likelihood = -2.39 Transmembrane 428 - 444 (425 - 445)
INTEGRAL Likelihood = -2.13 Transmembrane 383 - 399 (380 - 401)
INTEGRAL Likelihood = -1.97 Transmembrane 308 - 324 (304 - 327)
PERIPHERAL Likelihood = 0.37 modified ALOM score: 2.58

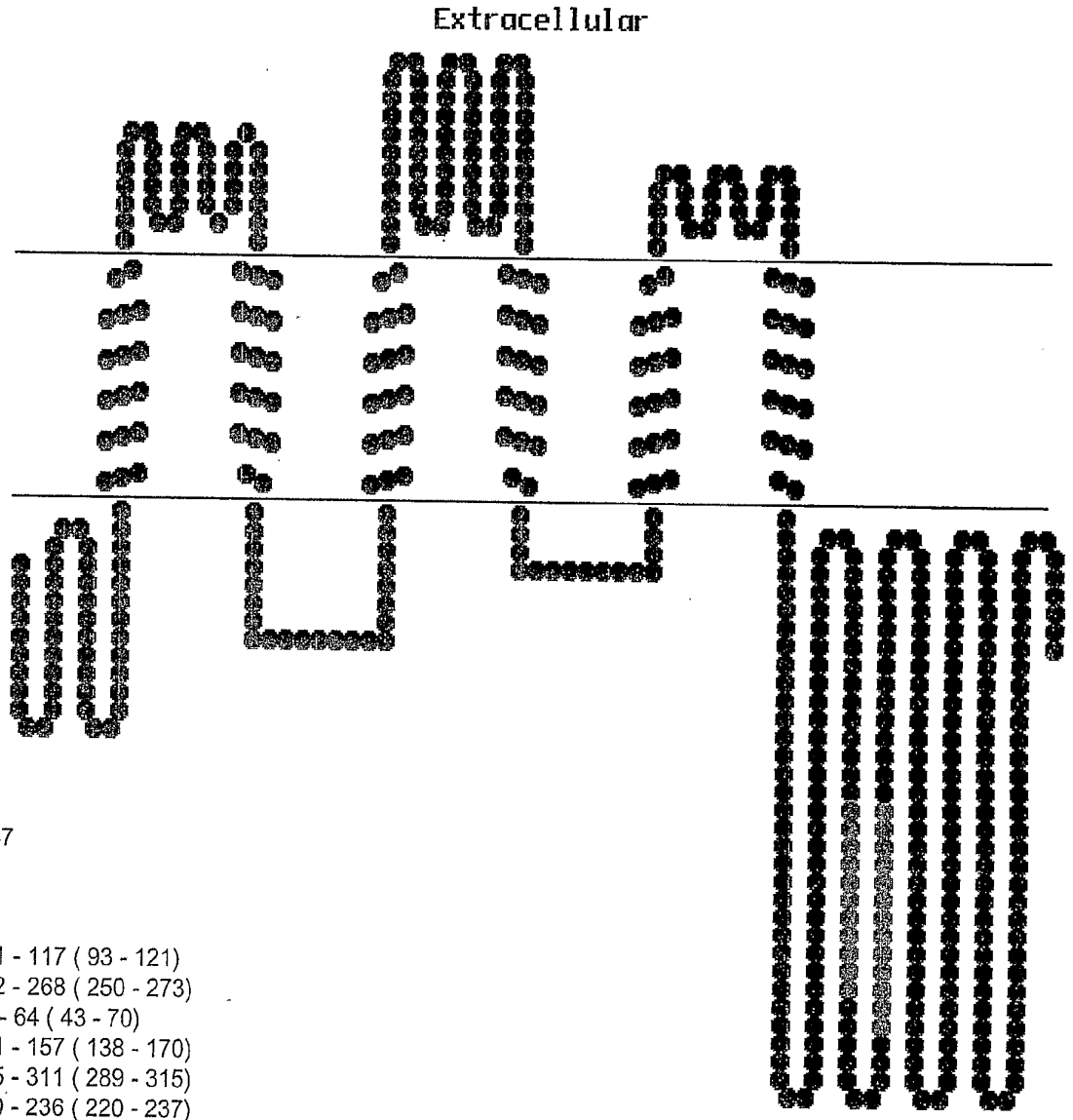
Rule: cytoplasmic membrane protein

FIG. 42



7 TM

GAS	SPY	FACS response
		Positive



Features: Membrane

PSORT prediction

Signal Score (-7.5): -3.98 Possible cleavage site: 47
>>> Seems to have no N-terminal signal seq.

count: 6 value: -8.86 threshold: 0.0
INTEGRAL Likelihood = -8.86 Transmembrane 101 - 117 (93 - 121)
INTEGRAL Likelihood = -7.54 Transmembrane 252 - 268 (250 - 273)
INTEGRAL Likelihood = -6.85 Transmembrane 48 - 64 (43 - 70)
INTEGRAL Likelihood = -5.57 Transmembrane 141 - 157 (138 - 170)
INTEGRAL Likelihood = -5.26 Transmembrane 295 - 311 (289 - 315)
INTEGRAL Likelihood = -0.53 Transmembrane 220 - 236 (220 - 237)
PERIPHERAL Likelihood = 2.28 modified ALOM score: 2.27

Rule: cytoplasmic membrane protein FIG. 43
GAS425, SPY 0184, FACS RESPONSE POSITIVE

6 TM

ORF	SPY	FACS response
gas493	spy0743	NOT CLONED

Features: Membrane

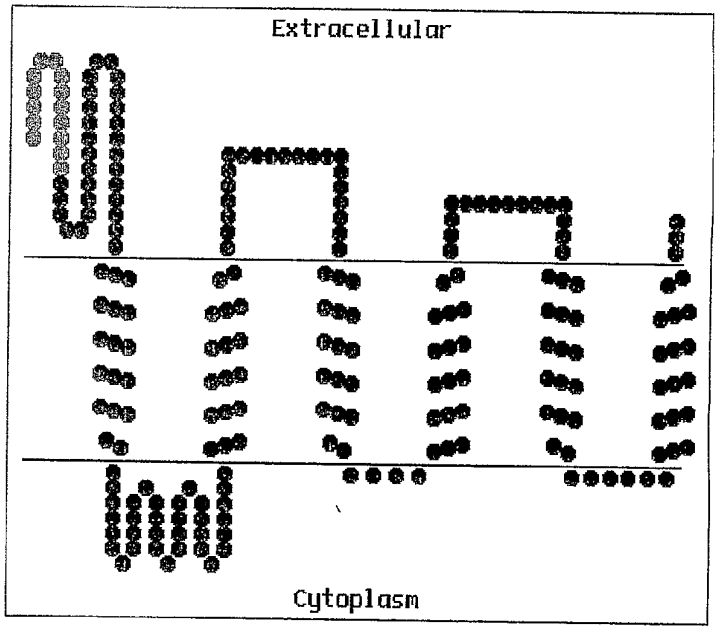
PSORT prediction

Signal Score (-7.5): -1.71 Possible cleavage site: 37
>>> Seems to have a cleavable N-term signal seq.

count: 6 value: -13.16 threshold: 0.0
INTEGRAL Likelihood =-13.16 Transmembrane 44 - 60 (39 - 71)
INTEGRAL Likelihood =-10.24 Transmembrane 94 - 110 (81 - 114)
INTEGRAL Likelihood = -7.64 Transmembrane 185 - 201 (179 - 207)
INTEGRAL Likelihood = -7.48 Transmembrane 132 - 148 (130 - 158)
INTEGRAL Likelihood = -2.76 Transmembrane 208 - 224 (204 - 225)
INTEGRAL Likelihood = -0.06 Transmembrane 153 - 169 (152 - 169)
PERIPHERAL Likelihood = 4.98 modified ALOM score: 3.13

Rule: cytoplasmic membrane protein

FIG. 44



6 TM

GAS	SPy	FACS response
gas469	SPy0645	NOT TESTED

Features: Membrane

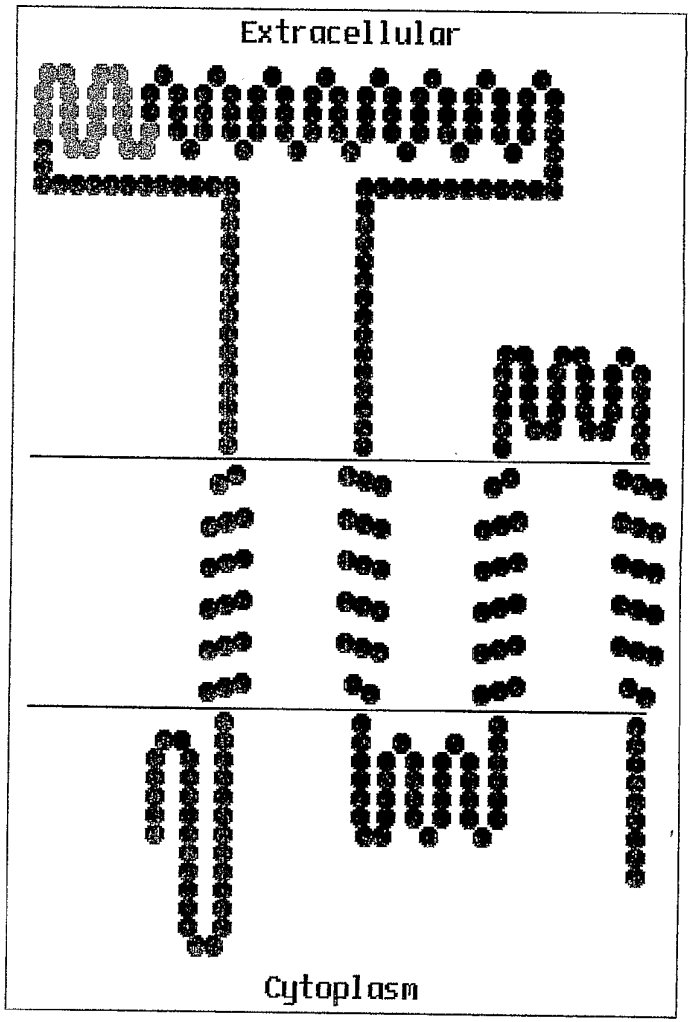
PSORT prediction

Signal Score (-7.5): 0.0600004 Possible cleavage site: 44
>>> Seems to have no N-terminal signal seq.

count: 4 value: -7.70 threshold: 0.0
INTEGRAL Likelihood = -7.70 Transmembrane 188 - 204 (182 - 212)
INTEGRAL Likelihood = -6.74 Transmembrane 32 - 48 (23 - 51)
INTEGRAL Likelihood = -5.52 Transmembrane 287 - 303 (281 - 307)
INTEGRAL Likelihood = -1.49 Transmembrane 239 - 255 (238 - 256)
PERIPHERAL Likelihood = 11.40 modified ALOM score: 2.04

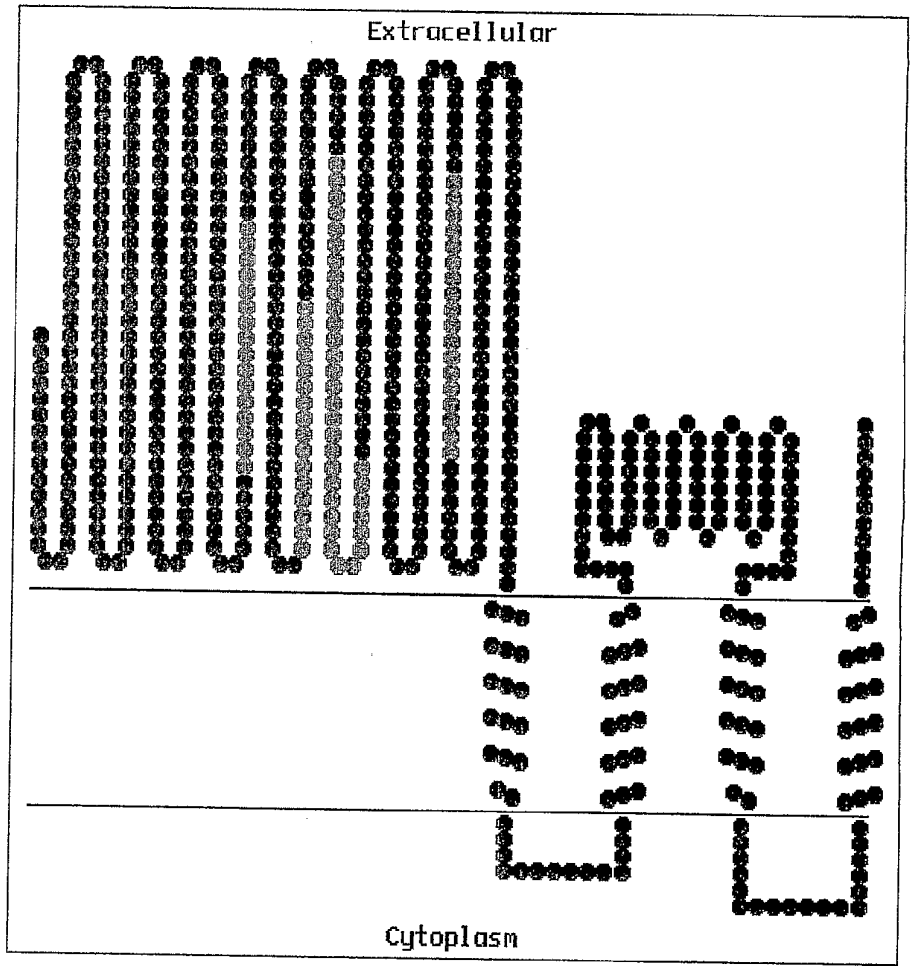
Rule: cytoplasmic membrane protein

FIG. 45



4 TM

GAS	SPY	FACS response
		Positive



Features: Membrane

PSORT prediction

Signal Score (-7.5): -2.04 Possible cleavage site: 20
>>> Seems to have a cleavable N-term signal seq.

count: 4 value: -10.51 threshold: 0.0
INTEGRAL Likelihood =-10.51 Transmembrane 529 - 545 (517 - 551)
INTEGRAL Likelihood =-10.30 Transmembrane 697 - 713 (693 - 719)
INTEGRAL Likelihood = -4.41 Transmembrane 560 - 576 (555 - 585)
INTEGRAL Likelihood = -0.32 Transmembrane 662 - 678 (662 - 678)
PERIPHERAL Likelihood = 0.95 modified ALOM score: 2.60

Rule: cytoplasmic membrane protein FIG. 46
GAS587, SPY 1315, FACS RESPONSE POSITIVE

4 TM

ʊ<ʊ	ʊn>	FACS response
gas645	spy2029	NOT CLONED

Features: Membrane

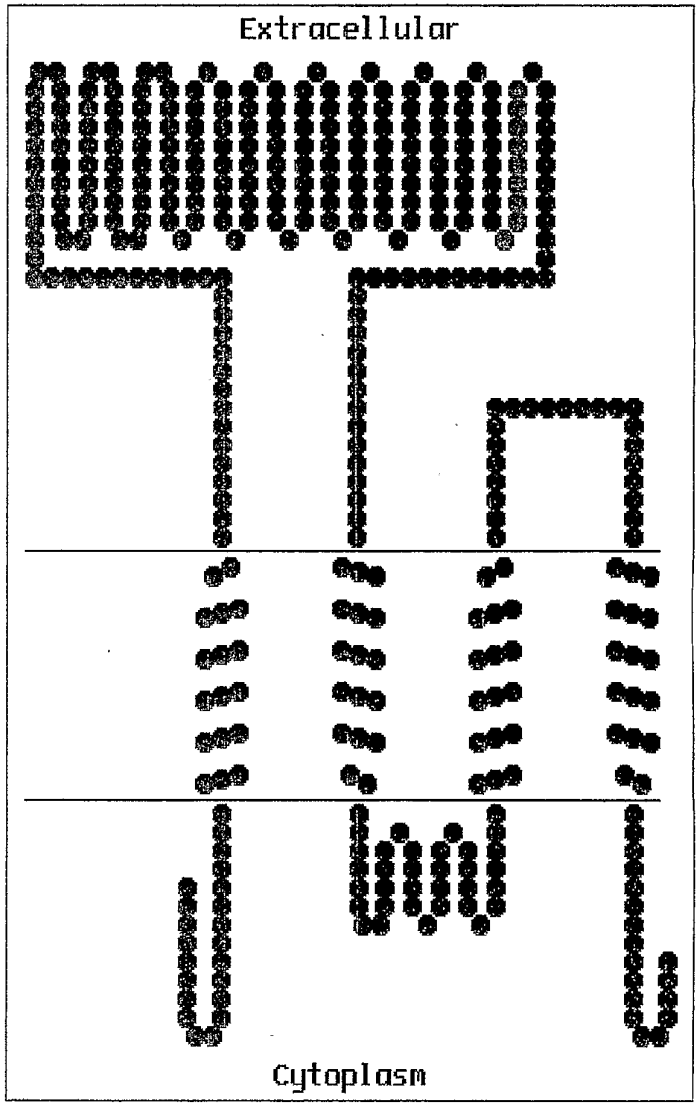
PSORT prediction

Signal Score (-7.5): -3.62 Possible cleavage site: 15
>>> Seems to have an uncleavable N-term signal seq

count: 4 value: -11.57 threshold: 0.0
INTEGRAL Likelihood =-11.57 Transmembrane 23 - 39 (16 - 43)
NTEGRAL Likelihood =-11.36 Transmembrane 371 - 387 (362 - 396)
INTEGRAL Likelihood = -8.12 Transmembrane 331 - 347 (324 - 360)
INTEGRAL Likelihood = -7.70 Transmembrane 280 - 296 (277 - 308)
PERIPHERAL Likelihood = 3.61 modified ALOM score: 2.81

Rule: cytoplasmic membrane protein

FIG. 47



4 TM

ORF	gene	FACS response
[redacted]	spy0277	Positive

Features: Membrane

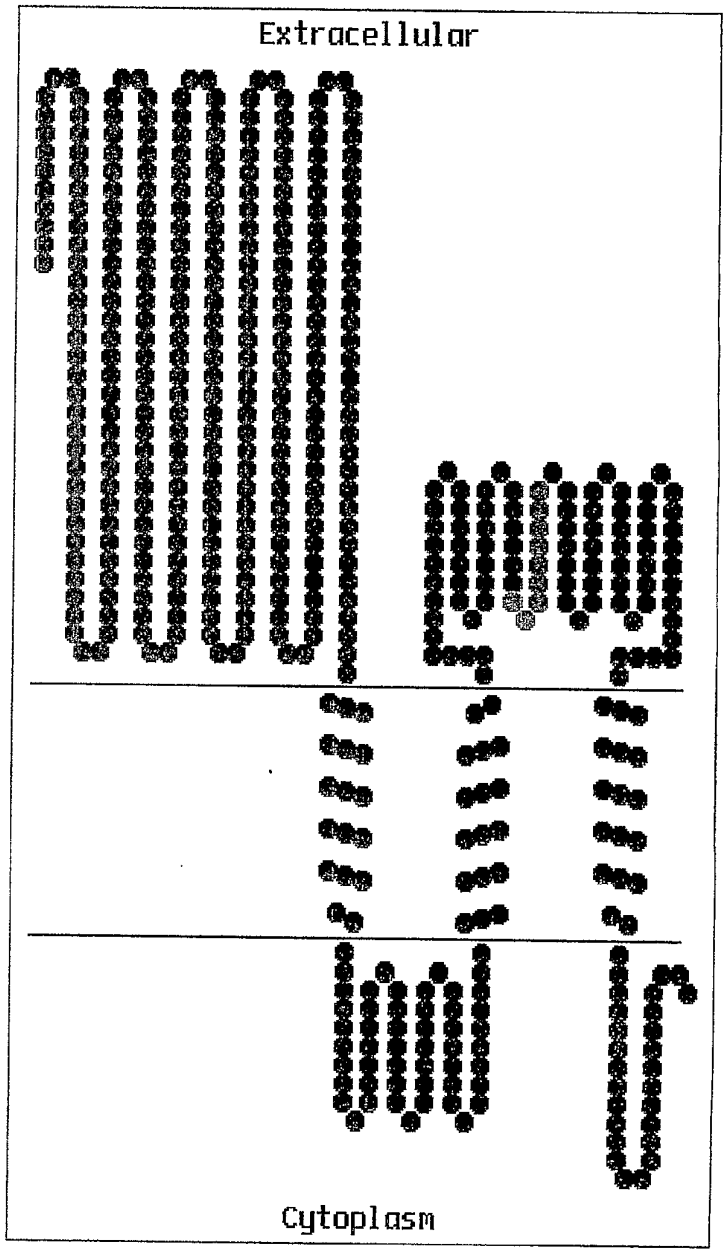
PSORT prediction

Signal Score (-7.5): 4.49 Possible cleavage site: 22
>>> Seems to have a cleavable N-term signal seq.

count: 3 value: -6.26 threshold: 0.0
INTEGRAL Likelihood = -6.26 Transmembrane 301 - 317 (297 - 321)
INTEGRAL Likelihood = -5.89 Transmembrane 479 - 495 (473 - 496)
INTEGRAL Likelihood = -1.12 Transmembrane 369 - 385 (369 - 385)
PERIPHERAL Likelihood = 1.32 modified ALOM score: 1.75

Rule: cytoplasmic membrane protein

FIG. 48
GAS433, SPY 0277, FACS RESPONSE POSITIVE



3 TM

GAS	SPY	FACS response
gas545	spy1740	NOT CLONED

Features: Membrane

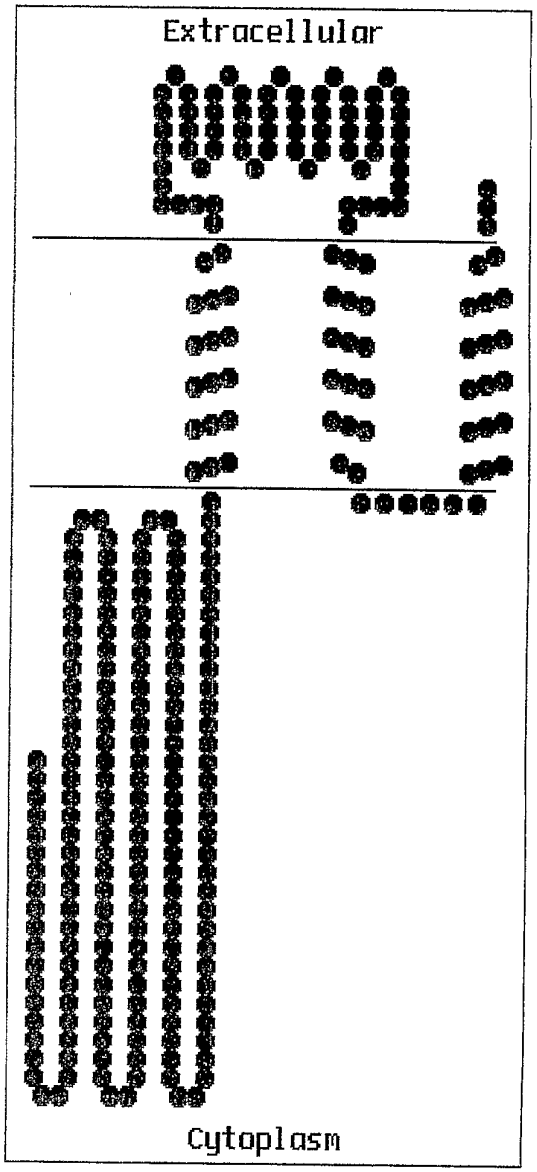
PSORT prediction

Signal Score (-7.5): -3.81 Possible cleavage site: 55
>>> Seems to have no N-terminal signal seq.

count: 3 value: -8.39 threshold: 0.0
INTEGRAL Likelihood = -8.39 Transmembrane 284 - 300 (279 - 302)
INTEGRAL Likelihood = -4.88 Transmembrane 261 - 277 (257 - 278)
INTEGRAL Likelihood = -4.51 Transmembrane 181 - 197 (180 - 198)
PERIPHERAL Likelihood = 0.79 modified ALOM score: 2.18

Rule: cytoplasmic membrane protein

FIG. 49



3 TM

GAS	SPY	FACS response
[redacted]	spy0351	ONLY FUSION 40

Features: Lipoprotein

PSORT prediction

Possible modific. site: 23
CRend: 6
Sequence Pattern: CVGR

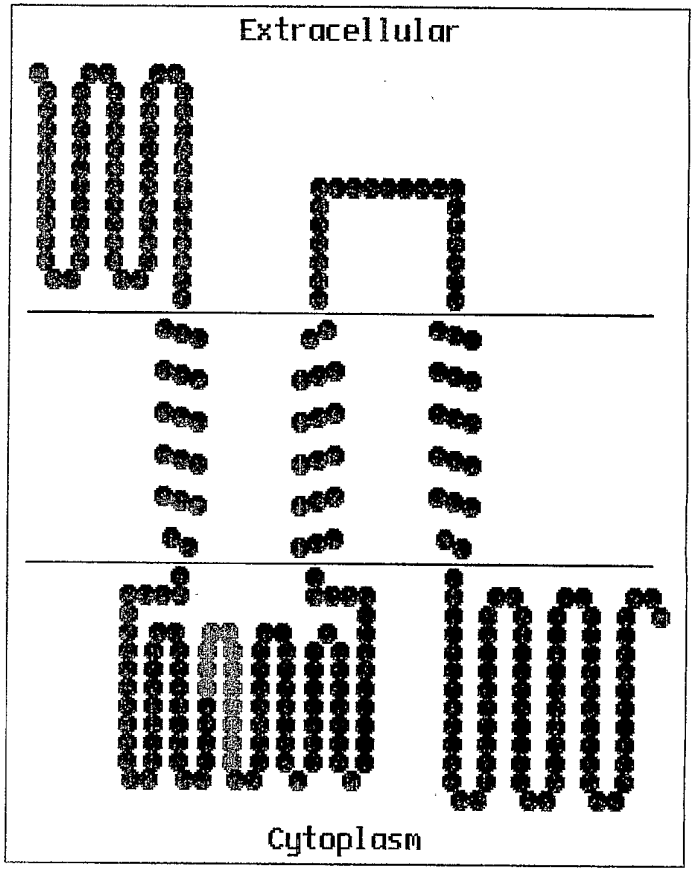
Signal Score (-7.5): -1.3 Possible cleavage site: 31
>>> May be a lipoprotein

count: 3 value: -9.55 threshold: 0.0
INTEGRAL Likelihood = -9.55 Transmembrane 62 - 78 (54 - 82)
INTEGRAL Likelihood = -2.81 Transmembrane 178 - 194 (177 - 195)
INTEGRAL Likelihood = -0.90 Transmembrane 216 - 232 (215 - 232)

PERIPHERAL Likelihood = 0.58 modified ALOM score: 2.41

Rule: cytoplasmic membrane protein

FIG. 50
GAS54, SPY 0351



3 TM Lipoprotein

GAS	SPY	FACS response
		Positive

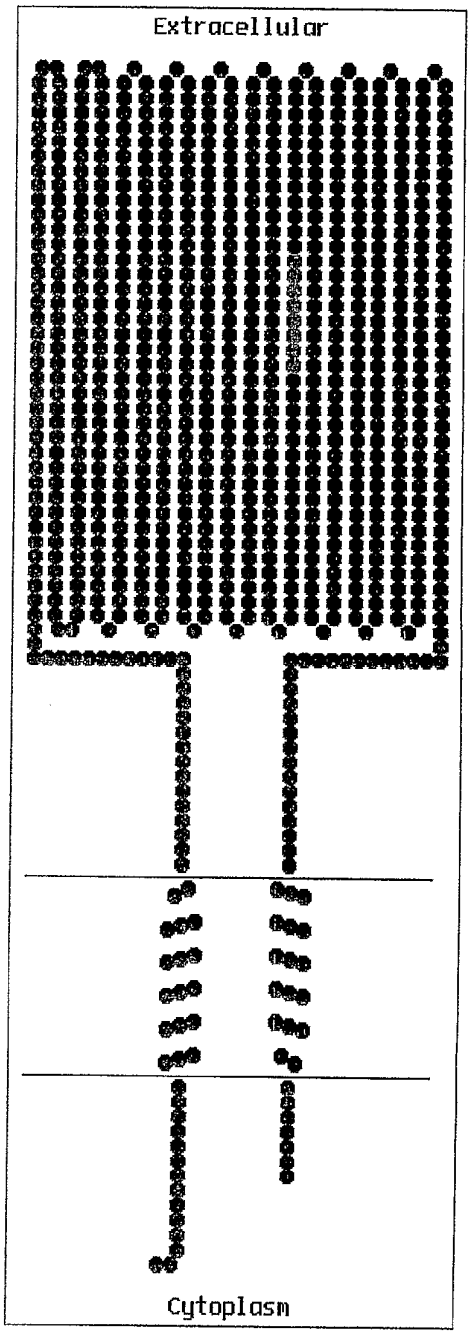
Features: Membrane

PSORT prediction

Signal Score (-7.5): 0.66 Possible cleavage site: 36
>>> Seems to have no N-terminal signal seq.

count: 2 value: -1.70 threshold: 0.0
INTEGRAL Likelihood = -1.70 Transmembrane 850 - 866 (850 - 866)
INTEGRAL Likelihood = -1.22 Transmembrane 15 - 31 (15 - 31)
PERIPHERAL Likelihood = 6.74 modified ALOM score: 0.84 Rule:
cytoplasmic membrane protein

FIG. 51
GAS40, SPY 0269



2 TM

cs	sp	FACS response
		Positive

Features: Membrane
Sortase

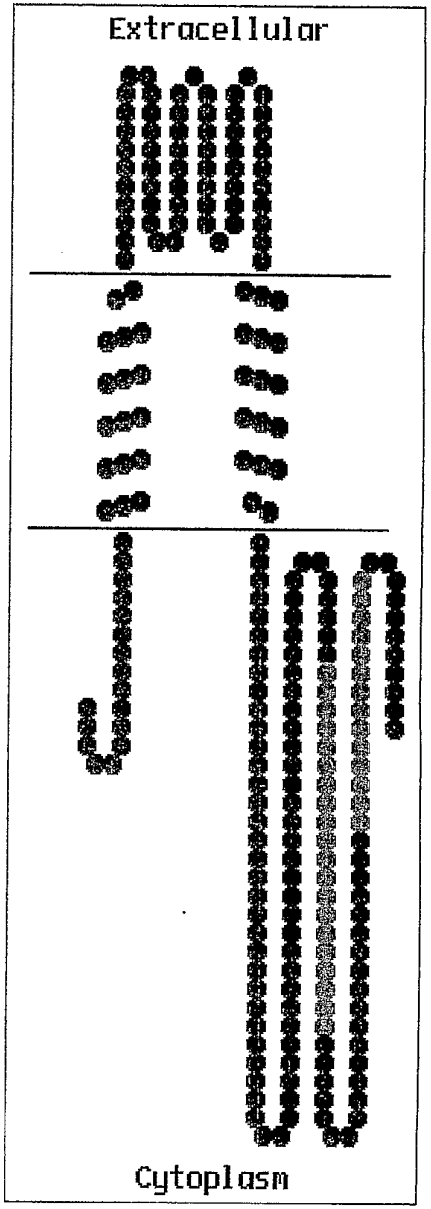
PSORT prediction

Signal Score (-7.5): -2.83 Possible cleavage site: 34
>>> Seems to have an uncleavable N-term signal seq

count: 2 value: -8.12 threshold: 0.0
INTEGRAL Likelihood = -8.12 Transmembrane 18 - 34 (13 - 38)
INTEGRAL Likelihood = -0.32 Transmembrane 94 - 110 (94 - 110)
PERIPHERAL Likelihood = 5.57 modified ALOM score: 2.12

Rule: cytoplasmic membrane protein

FIG. 52
GAS163, SPY 1154



2 TM

GAS	SP	FACS response
gas198	spy2184	Negative

Features: Membrane

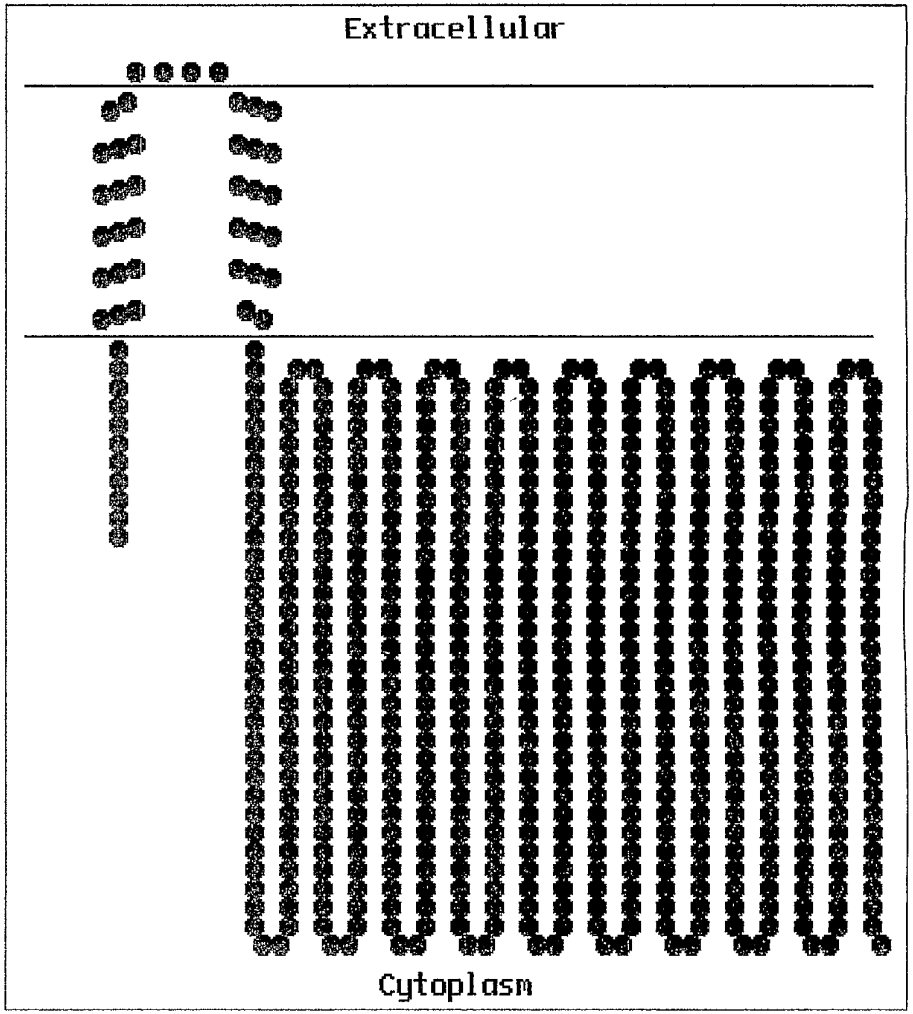
PSORT prediction

Signal Score (-7.5): -3.79 Possible cleavage site: 25
>>> Seems to have an uncleavable N-term signal seq

count: 2 value: -18.57 threshold: 0.0
INTEGRAL Likelihood =-18.57 Transmembrane 33 - 49 (6 - 56)
INTEGRAL Likelihood =-10.14 Transmembrane 12 - 28 (6 - 32)
PERIPHERAL Likelihood = 2.44 modified ALOM score: 4.21

Rule: cytoplasmic membrane protein

FIG. 53



2 TM

GAS	SAY	FACS response
gas224	spy1044	NOT CLONED

Features: Membrane

PSORT prediction

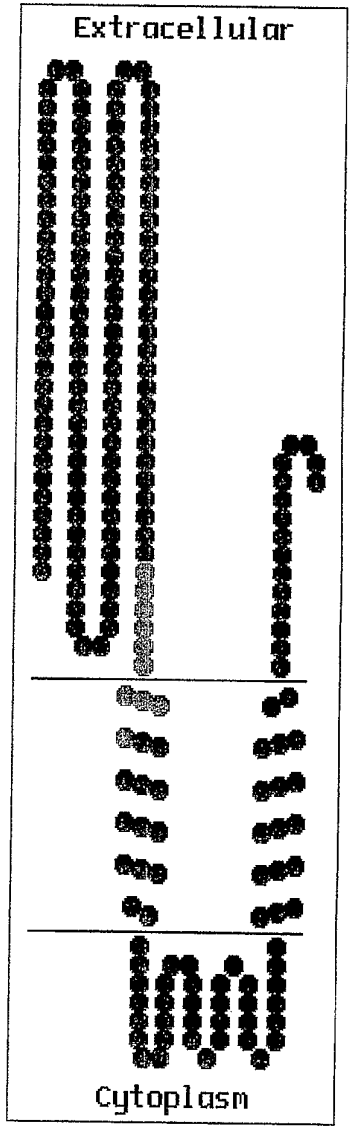
Signal Score (-7.5): -0.659999 Possible cleavage site: 18

>>> Seems to have a cleavable N-term signal seq.

count: 2 value: -7.32 threshold: 0.0
INTEGRAL Likelihood = -7.32 Transmembrane 126 - 142 (118 - 145)
INTEGRAL Likelihood = -6.90 Transmembrane 178 - 194 (177 - 203)
PERIPHERAL Likelihood = 0.79 modified ALOM score: 1.96

Rule: cytoplasmic membrane protein

FIG. 54



2 TM

GAS	SAG	FACS response
gas500	spy1410	NOT CLONED

Features: Membrane

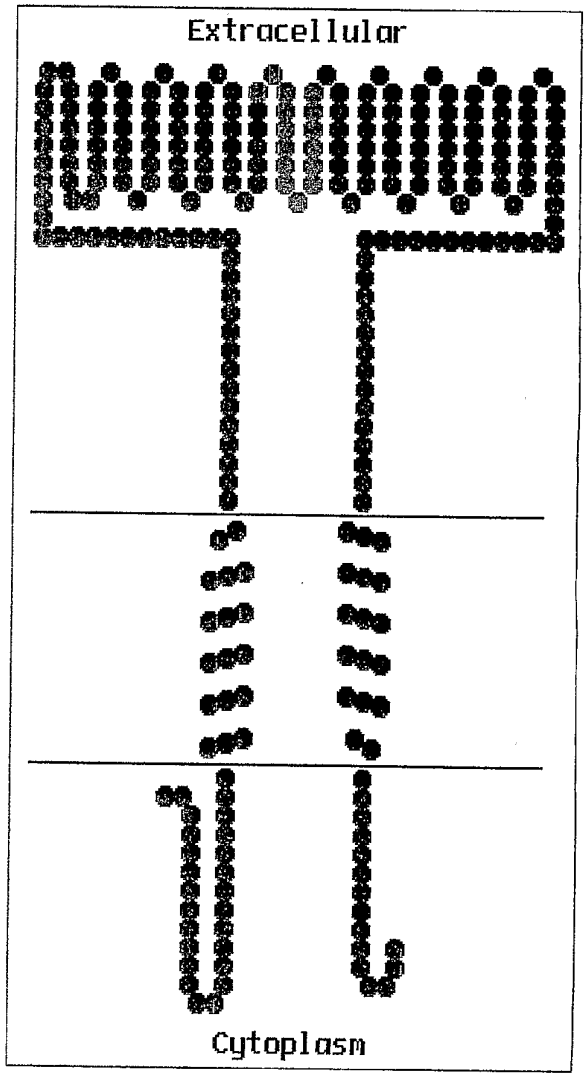
PSORT prediction

Signal Score (-7.5): -1.18 Possible cleavage site: 49
>>> Seems to have no N-terminal signal seq.

count: 2 value: -11.83 threshold: 0.0
INTEGRAL Likelihood =-11.83 Transmembrane 241 - 257 (234 - 266)
INTEGRAL Likelihood = -4.41 Transmembrane 27 - 43 (26 - 44)
PERIPHERAL Likelihood = 2.28 modified ALOM score: 2.87

Rule: cytoplasmic membrane protein

FIG. 55



2 TM

GAS	SPY	FACS response
		Negative

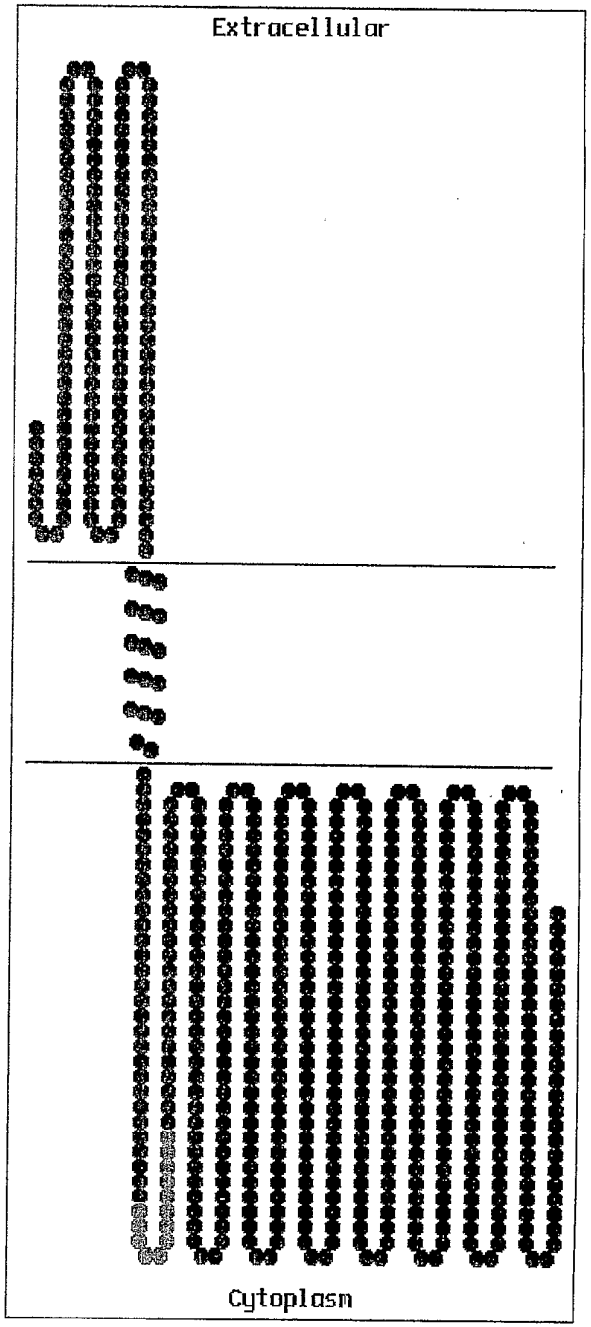
Features: Membrane

PSORT prediction

Signal Score (-7.5): -2.36
Possible cleavage site: 38
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -7.38 threshold: 0.0
INTEGRAL Likelihood = -7.38 Transmembrane 138 - 154 (132 - 158)
PERIPHERAL Likelihood = 2.23 modified
ALOM score: 1.98
Rule: cytoplasmic membrane protein

FIG. 56
GAS4, SPY 0015



GAS	SPY	FACS response
		Positive

Features: Membrane

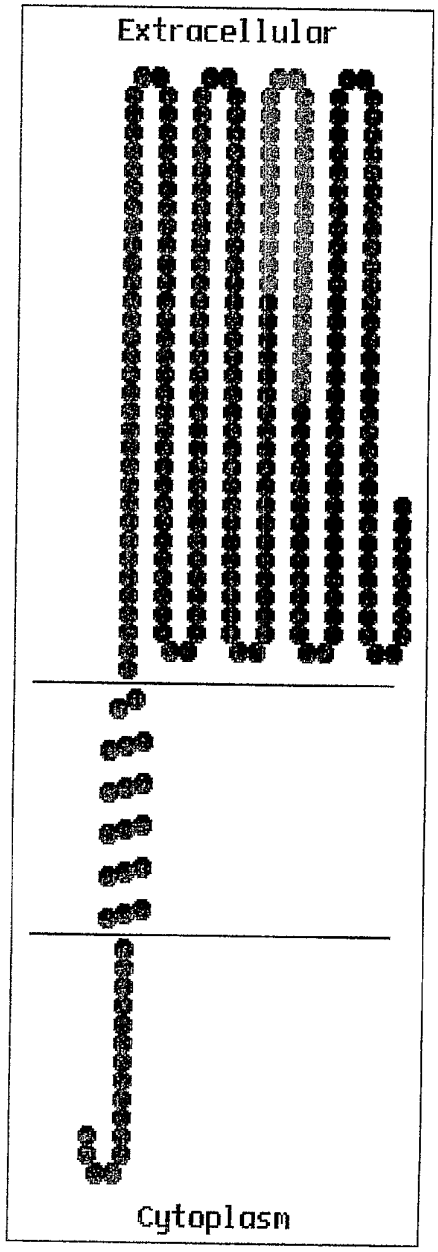
PSORT prediction

Signal Score (-7.5): -0.17 Possible cleavage site: 30
>>> Seems to have no N-terminal signal seq.

count: 1 value: -6.16 threshold: 0.0
INTEGRAL Likelihood = -6.16 Transmembrane 17 - 33 (15 - 35)
PERIPHERAL Likelihood = 10.61 modified ALOM score: 1.73

Rule: cytoplasmic membrane protein

FIG. 57
GAS72, SPY 0903



ORF	Name	FACS response
gas152	spy0802	Negative

Features: Membrane

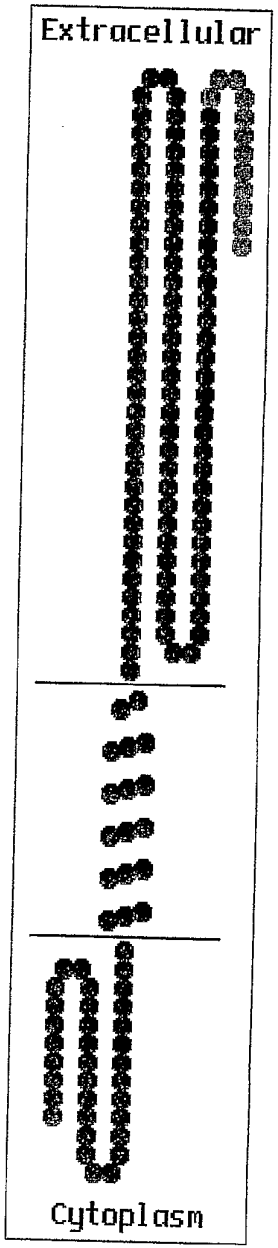
PSORT prediction

Signal Score (-7.5): -4.12 Possible cleavage site: 43
>>> Seems to have no N-terminal signal seq.

count: 1 value: -15.97 threshold: 0.0
INTEGRAL Likelihood =-15.97 Transmembrane 35 - 51 (25 - 58)
PERIPHERAL Likelihood = 5.20 modified ALOM score: 3.69

Rule: cytoplasmic membrane protein

FIG. 58
GAS152, SPY 0802



		FACS response
		Positive

Features: Membrane

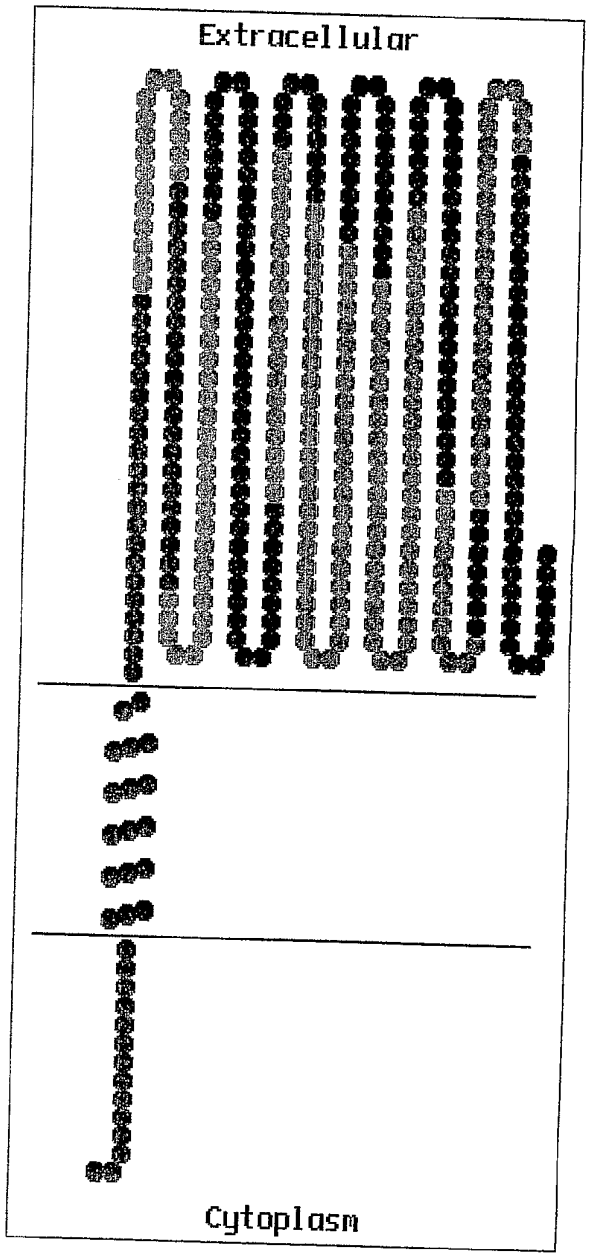
PSORT prediction

Signal Score (-7.5): -6.52 Possible cleavage site: 42
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -9.61 threshold: 0.0
INTEGRAL Likelihood = -9.61 Transmembrane 15 - 31 (11 - 36)
PERIPHERAL Likelihood = 1.32 modified ALOM score: 2.42

Rule: cytoplasmic membrane protein

FIG. 59
GAS157, SPY 0836



GAS	SPY	FACS response
		NOT CLONED

Features: Membrane

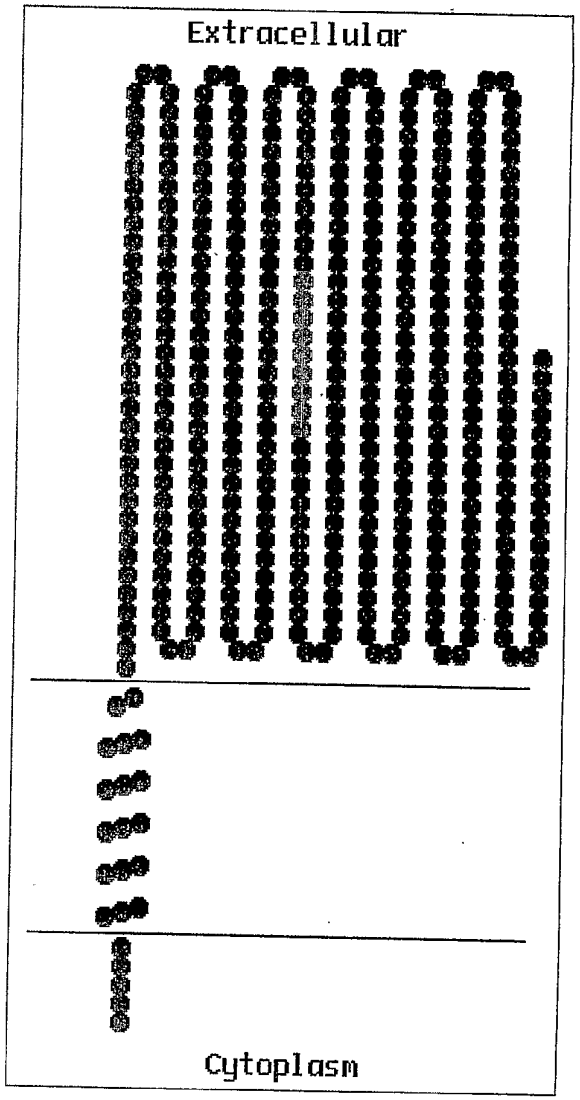
PSORT prediction

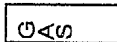
Signal Score (-7.5): -2.66 Possible cleavage site: 22
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -12.58 threshold: 0.0
INTEGRAL Likelihood =-12.58 Transmembrane 6 - 22 (1 - 27)
PERIPHERAL Likelihood = 4.61 modified ALOM score: 3.02

Rule: cytoplasmic membrane protein

FIG. 60
GAS168, SPY 1370



		FACS response
		Positive

Features: Membrane

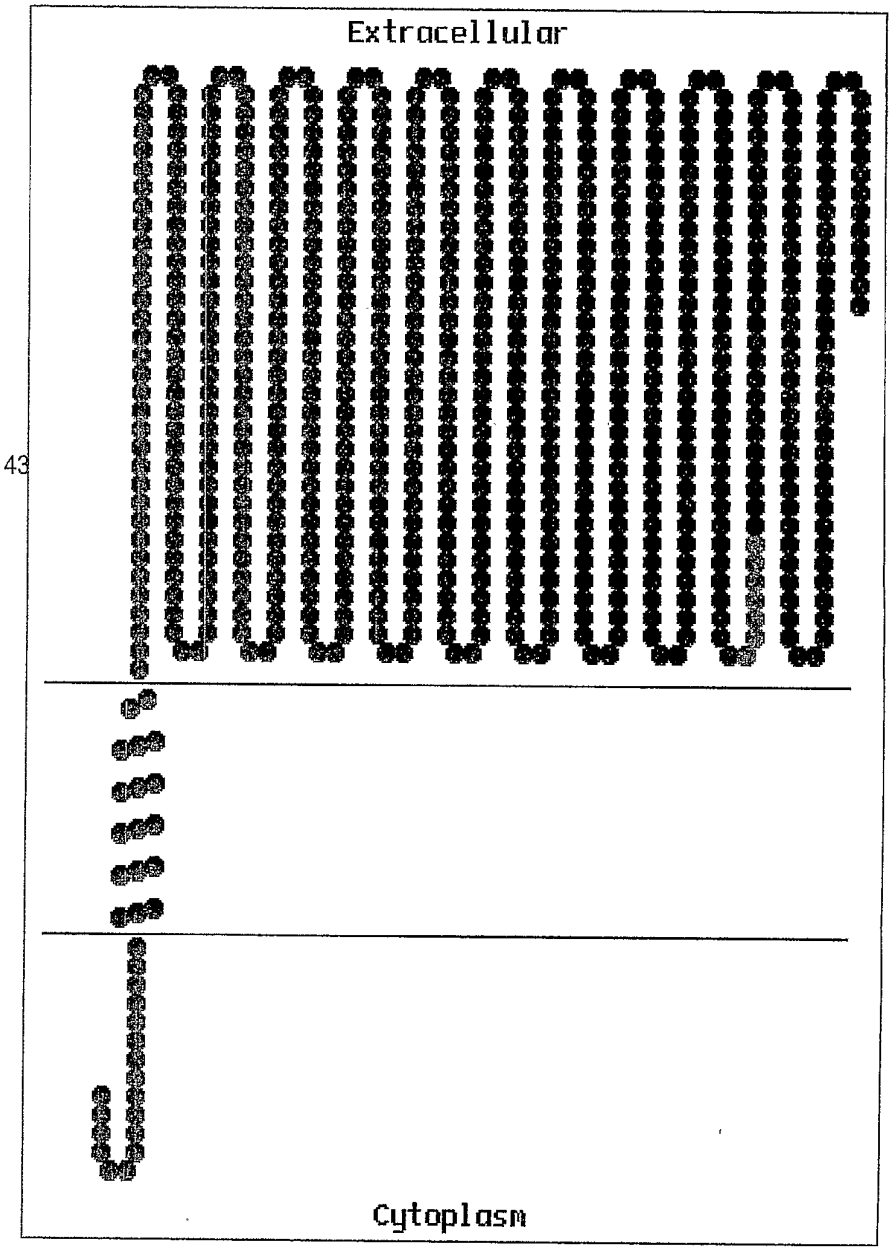
PSORT prediction

Signal Score (-7.5): -2.9 Possible cleavage site: 41
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -13.96 threshold: 0.0
INTEGRAL Likelihood =-13.96 Transmembrane 19 - 35 (9 - 43
PERIPHERAL Likelihood = 4.35 modified ALOM score: 3.29

Rule: cytoplasmic membrane protein

FIG. 61
GAS177, SPY 1649



ORF	ORF	FACS response
gas193	spy2025	Negative

Features: Membrane

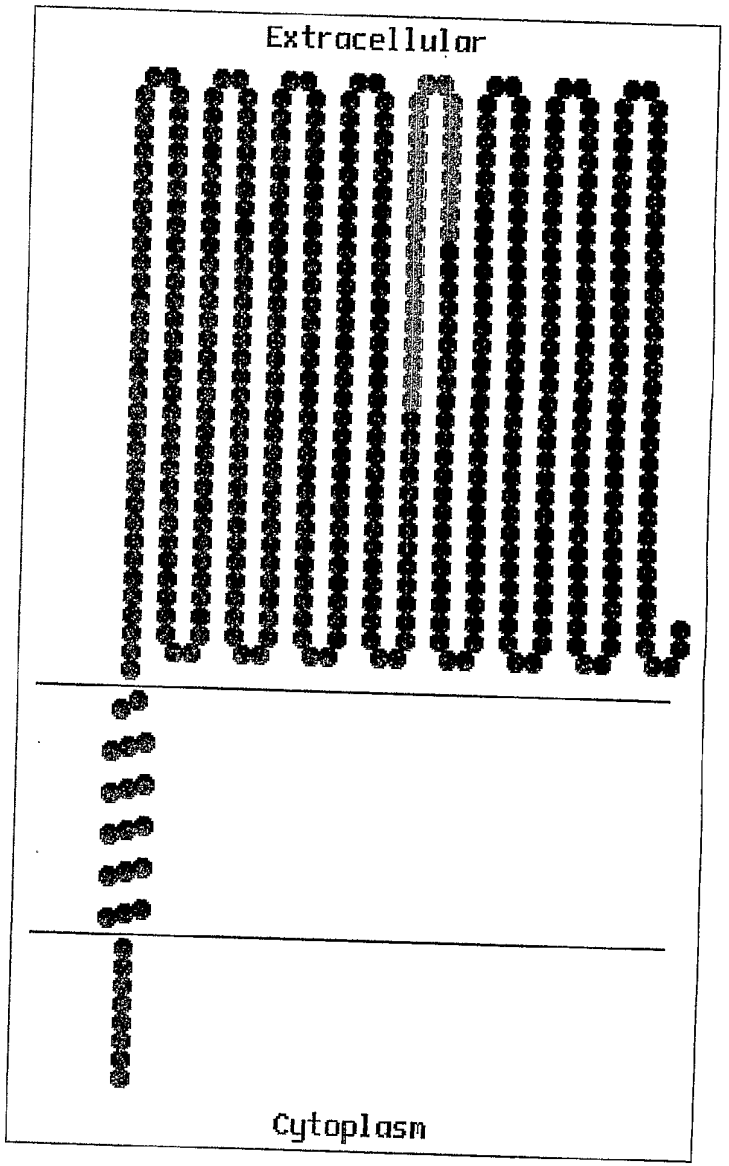
PSORT prediction

Signal Score (-7.5): -3.83 Possible cleavage site: 19
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -3.77 threshold: 0.0
INTEGRAL Likelihood = -3.77 Transmembrane 9 - 25 (5 - 27)
PERIPHERAL Likelihood = 3.29 modified ALOM score: 1.25

Rule: cytoplasmic membrane protein

FIG. 62



		FACS response
		Positive

Features: Membrane

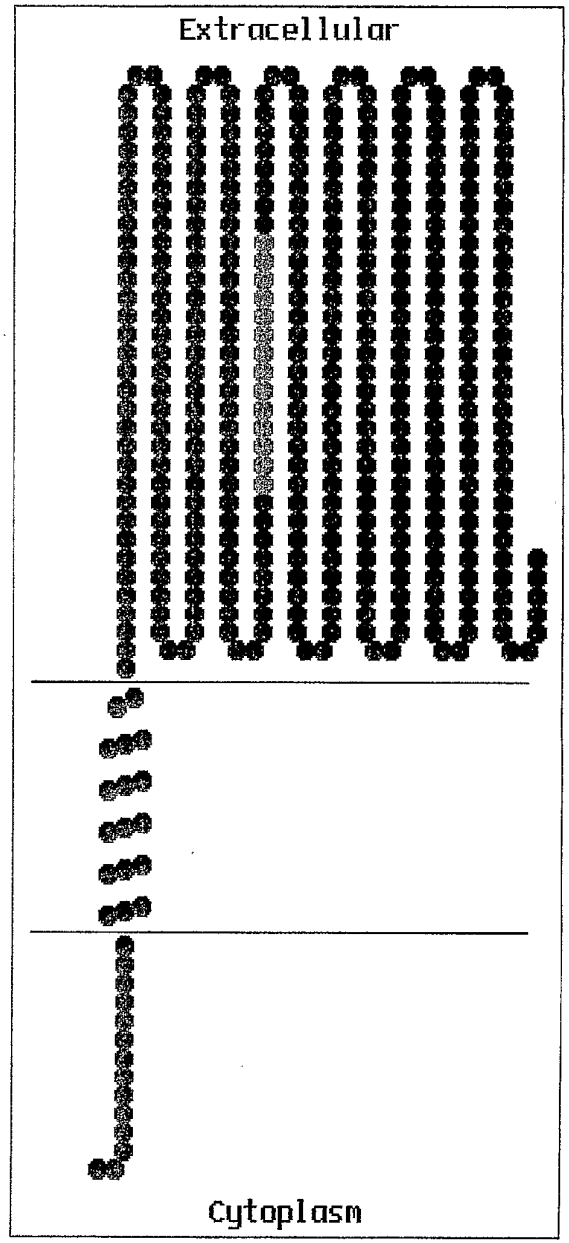
PSORT prediction

Signal Score (-7.5): -4.21 Possible cleavage site: 26
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -2.60 threshold: 0.0
INTEGRAL Likelihood = -2.60 Transmembrane 15 - 31 (12 - 32)
PERIPHERAL Likelihood = 9.02 modified ALOM score: 1.02

Rule: cytoplasmic membrane protein

FIG. 63
GAS194, SPY 2032



GAS	SPY	FACS response
		Negative

Features: Membrane

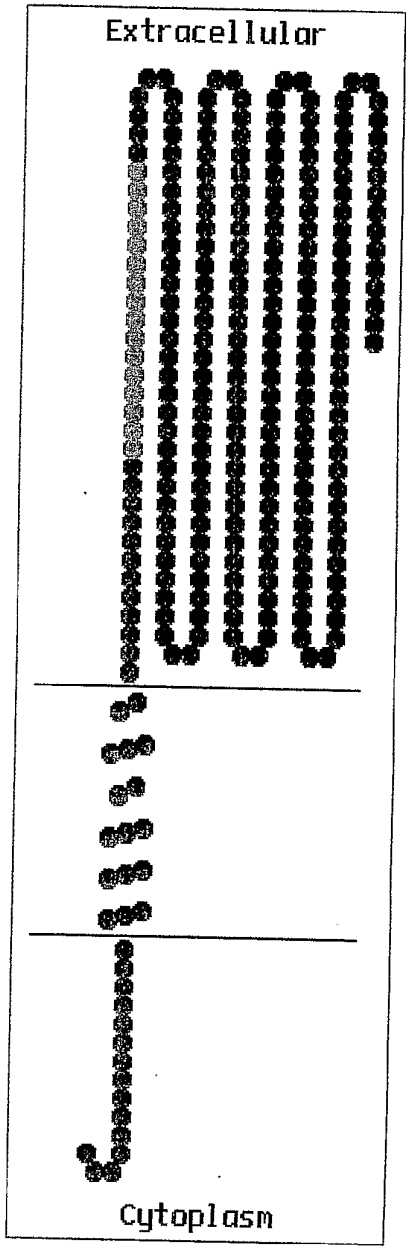
PSORT prediction

Signal Score (-7.5): -1.6 Possible cleavage site: 28
>>> Seems to have no N-terminal signal seq.

count: 1 value: -1.38 threshold: 0.0
INTEGRAL Likelihood = -1.38 Transmembrane 16 - 32 (16 - 32)
PERIPHERAL Likelihood = 7.32 modified ALOM score: 0.78

Rule: cytoplasmic membrane protein

FIG. 64
GAS195, SPY 2043



GAS	SPY	FACS response
gas149	spy0780	Positive

Features: Membrane

PSORT prediction

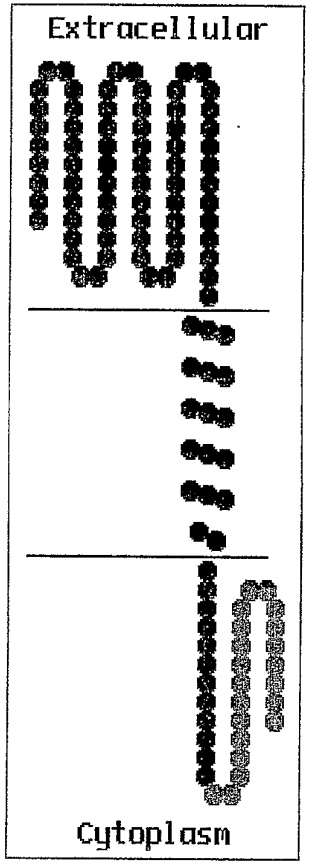
Signal Score (-7.5): 0.0299997 Possible cleavage site: 28

>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -5.95 threshold: 0.0
INTEGRAL Likelihood = -5.95 Transmembrane 71 - 87 (67 - 90)
PERIPHERAL Likelihood = 3.18 modified ALOM score: 1.69

Rule: cytoplasmic membrane protein

FIG. 65
GAS149, SPY 0780



GAS	SPY	FACS response
gas149	spy0780	Positive

Features: Membrane

PSORT prediction

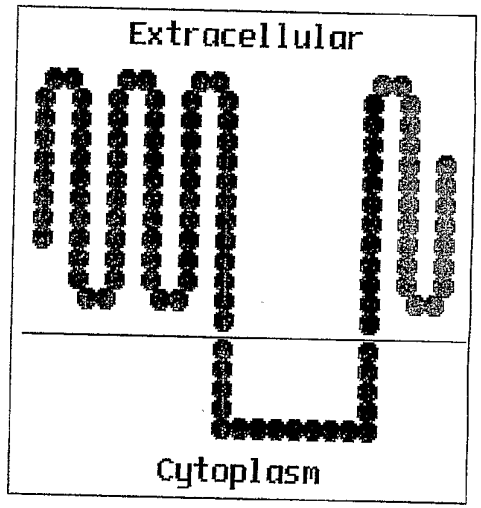
Signal Score (-7.5): 0.0299997 Possible cleavage site: 28

>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -5.95 threshold: 0.0
INTEGRAL Likelihood = -5.95 Transmembrane 71 - 87 (67 - 90)
PERIPHERAL Likelihood = 3.18 modified ALOM score: 1.69

Rule: cytoplasmic membrane protein

FIG. 66
GAS149, SPY 0780



GAS	SP	FACS response
		Positive

Features: Membrane

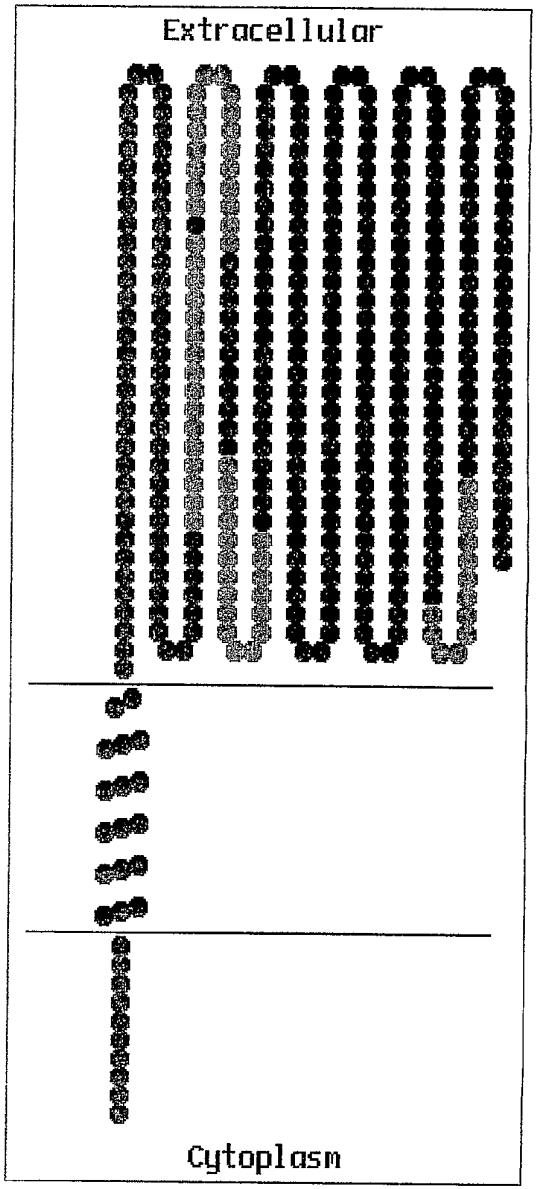
PSORT prediction

Signal Score (-7.5): -4.39 Possible cleavage site: 24
>>> Seems to have an uncleavable N-term signal seq

count: 1 value: -8.76 threshold: 0.0
INTEGRAL Likelihood = -8.76 Transmembrane 11 - 27 (6 - 31)
PERIPHERAL Likelihood = 3.29 modified ALOM score: 2.25

Rule: cytoplasmic membrane protein

FIG. 67
GAS201, SPY 2216



ʊ<ʊ	ʊɒ>	FACS response
gas251	spy1520	Positive

Features: Membrane

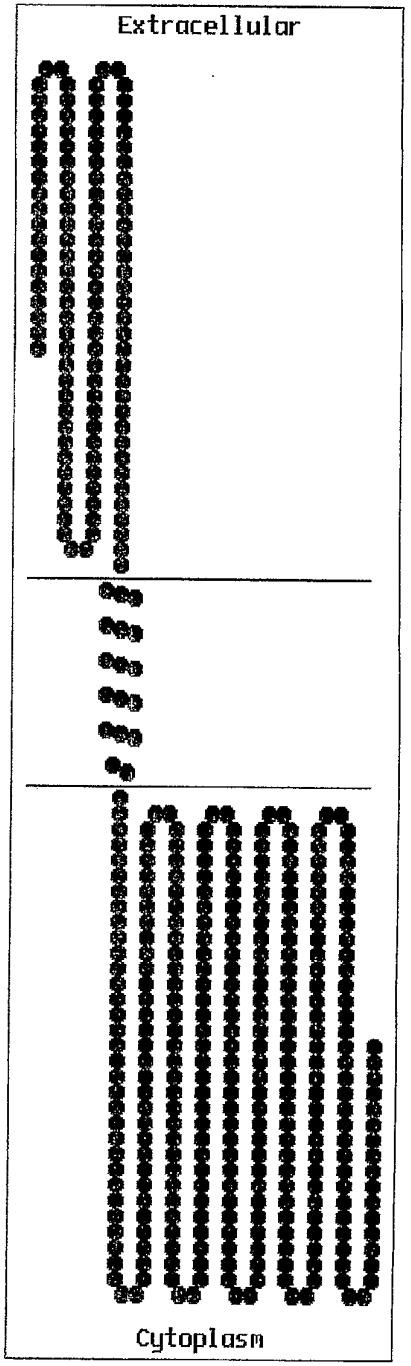
PSORT prediction

Signal Score (-7.5): -1.75 Possible cleavage site: 56
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -1.81 threshold: 0.0
INTEGRAL Likelihood = -1.81 Transmembrane 117 - 133 (117 - 133)
PERIPHERAL Likelihood = 3.13 modified ALOM score: 0.86

Rule: cytoplasmic membrane protein

FIG. 68
GAS251, SPY 1520



GAS	SP	FACS response
gas259	spy1586	NOT CLONED

Features: Membrane

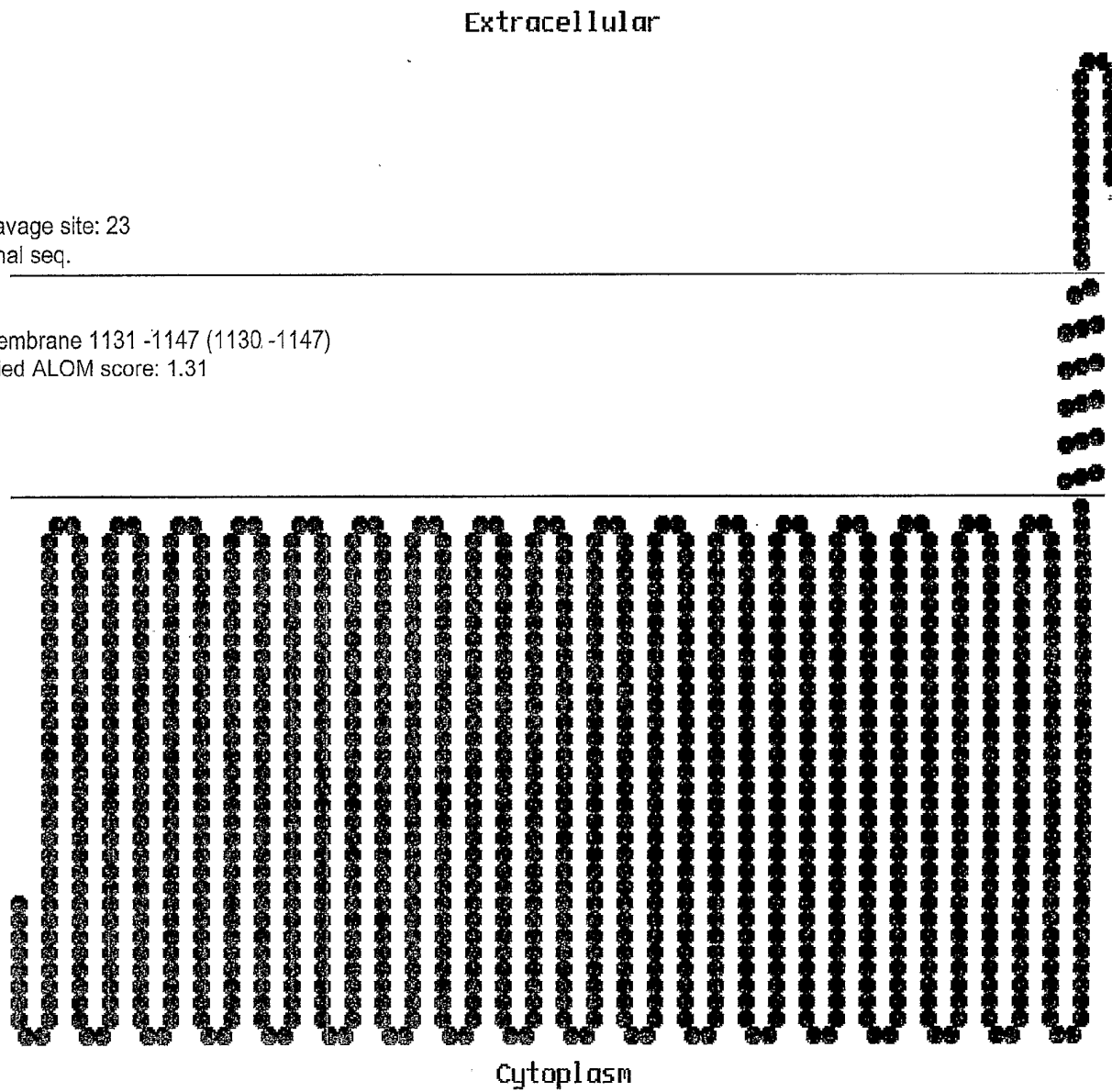
PSORT prediction

Signal Score (-7.5): -7.91 Possible cleavage site: 23
>>> Seems to have no N-terminal signal seq.

count: 1 value: -4.04 threshold: 0.0
INTEGRAL Likelihood = -4.04 Transmembrane 1131 -1147 (1130 -1147)
PERIPHERAL Likelihood = 1.11 modified ALOM score: 1.31

Rule: cytoplasmic membrane protein

FIG. 69
GAS259, SPY 1586



		FACS response
		NOT CLONED

Features: Membrane

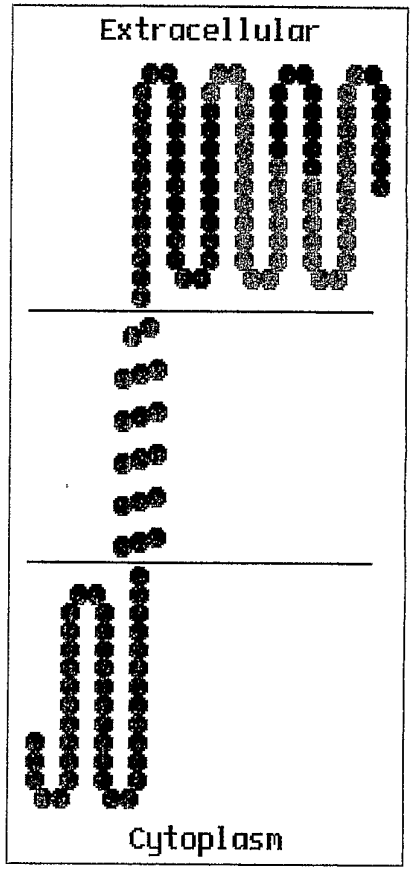
PSORT prediction

Signal Score (-7.5): 1.3 Possible cleavage site: 57
>>> Seems to have no N-terminal signal seq.

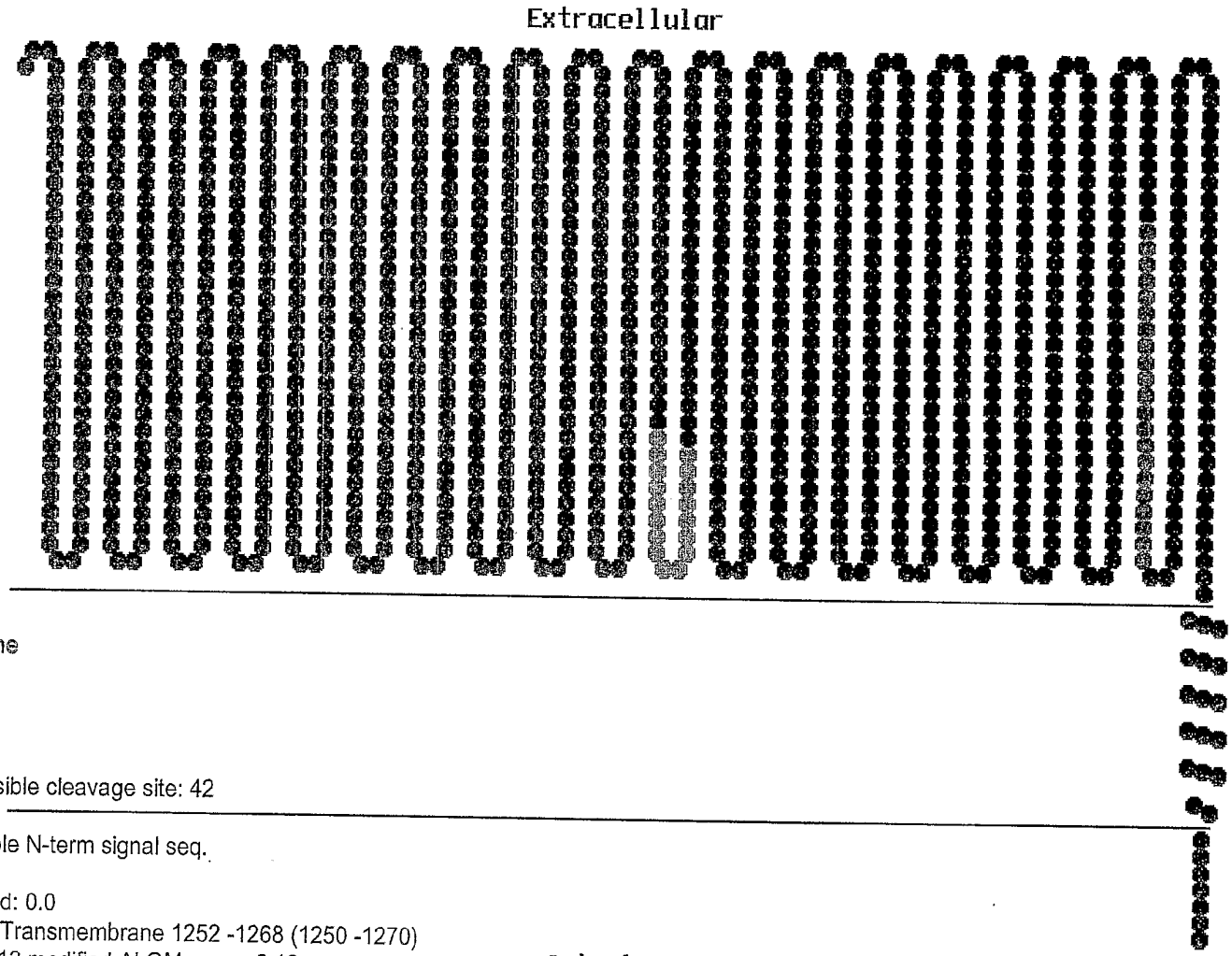
count: 1 value: -5.15 threshold: 0.0
INTEGRAL Likelihood = -5.15 Transmembrane 42 - 58 (41 - 60)
PERIPHERAL Likelihood = 2.28 modified ALOM score: 1.53

Rule: cytoplasmic membrane protein

FIG. 70
GAS264, SPY 1686



GAS	SPY	FACS response
		Positive



Features: Membrane

PSORT prediction

Signal Score (-7.5): 0.13 Possible cleavage site: 42

>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -8.17 threshold: 0.0
NTEGRAL Likelihood = -8.17 Transmembrane 1252 -1268 (1250 -1270)
PERIPHERAL Likelihood = 3.13 modified ALOM score: 2.13

Rule: cytoplasmic membrane protein FIG. 71
GAS268, SPY 1798

GAS	SPY	FACS response
		Positive

Features: Membrane

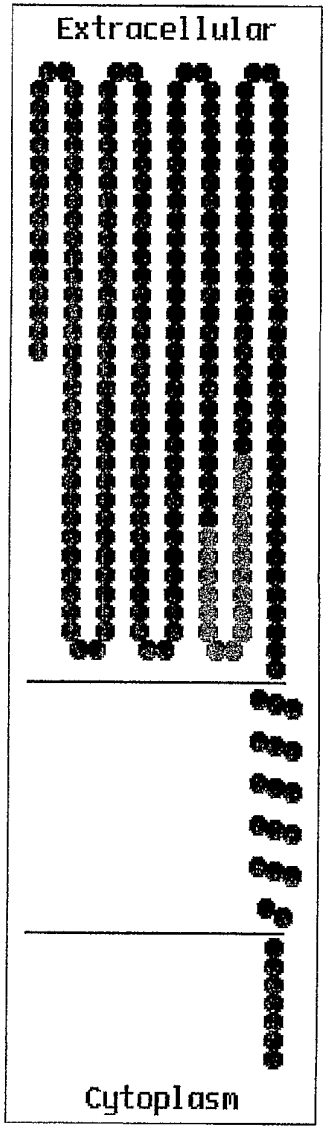
PSORT prediction

Signal Score (-7.5): -0.82 Possible cleavage site: 31
>>> Seems to have a cleavable N-term signal seq.

count: 1 value: -10.77 threshold: 0.0
INTEGRAL Likelihood =-10.77 Transmembrane 242 - 258 (238 - 262)
PERIPHERAL Likelihood = 9.39 modified ALOM score: 2.65

Rule: cytoplasmic membrane protein

FIG. 72
GAS277, SPY 1939



		FACS response
		Positive

Features: Membrane

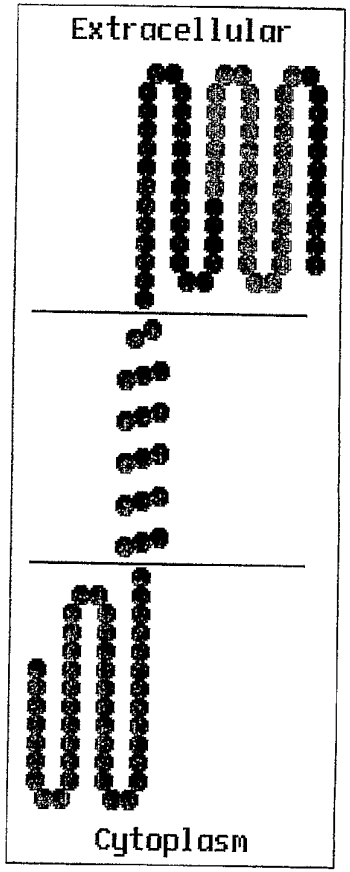
PSORT prediction

Signal Score (-7.5): -0.93 Possible cleavage site: 55
>>> Seems to have no N-terminal signal seq.

count: 1 value: -6.64 threshold: 0.0
INTEGRAL Likelihood = -6.64 Transmembrane 46 - 62 (43 - 65)
PERIPHERAL Likelihood = 11.14 modified ALOM score: 1.83

Rule: cytoplasmic membrane protein

FIG. 73
GAS282, SPY 2033



GAS	SPY	FACS response
gas299	spy1188	NOT CLONED

Features: Membrane

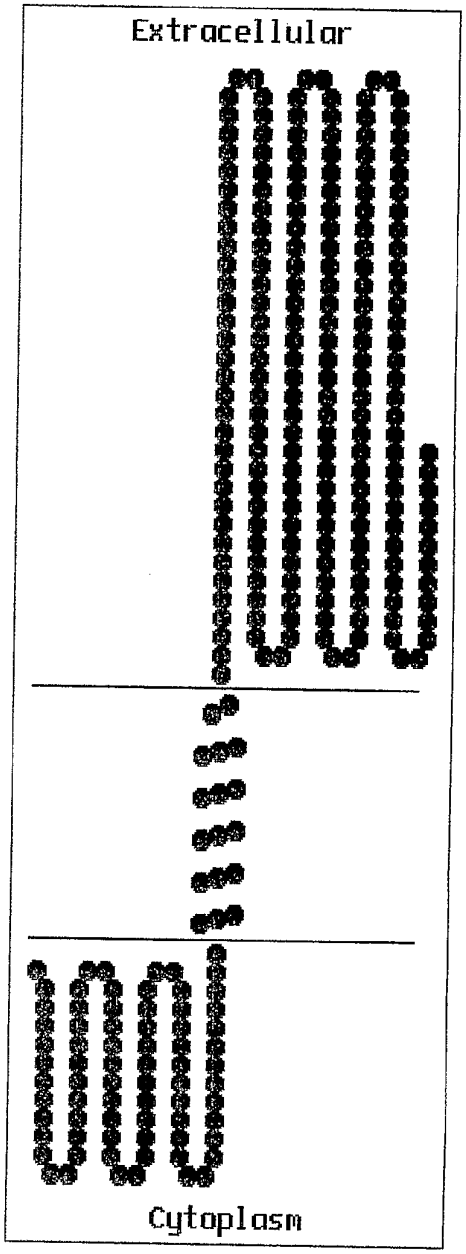
PSORT prediction

Signal Score (-7.5): 4.07 Possible cleavage site: 16
>>> Seems to have no N-terminal signal seq.

count: 1 value: -1.65 threshold: 0.0
INTEGRAL Likelihood = -1.65 Transmembrane 74 - 90 (74 - 90)
PERIPHERAL Likelihood = 2.65 modified ALOM score: 0.83

Rule: cytoplasmic membrane protein

FIG. 74
GAS299, SPY 1188



GAS	SPY	FACS response
gas262	spy1643	NOT CLONED

Features: Membrane

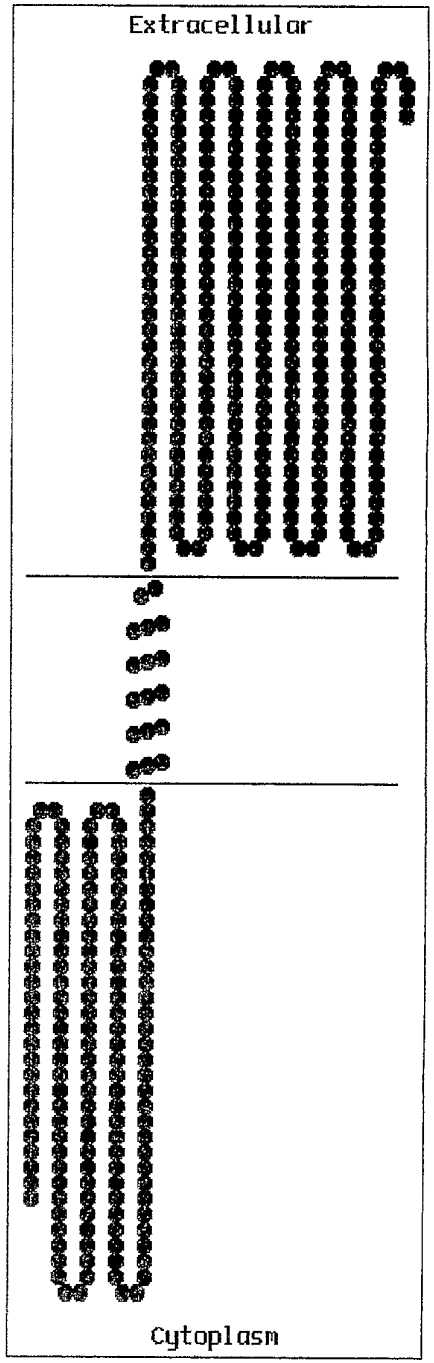
PSORT prediction

Signal Score (-7.5): -9.4 Possible cleavage site: 42
>>> Seems to have no N-terminal signal seq.

count: 1 value: -14.70 threshold: 0.0
INTEGRAL Likelihood =-14.70 Transmembrane 156 - 172 (151 - 178)
PERIPHERAL Likelihood = 0.79 modified ALOM score: 3.44

Rule: cytoplasmic membrane protein

FIG. 75
GAS262, SPY 1643



ᑐᐊᔪ	ᔪᓇᐳ	FACS response
gas405	spy1028	Positive

Features: Membrane

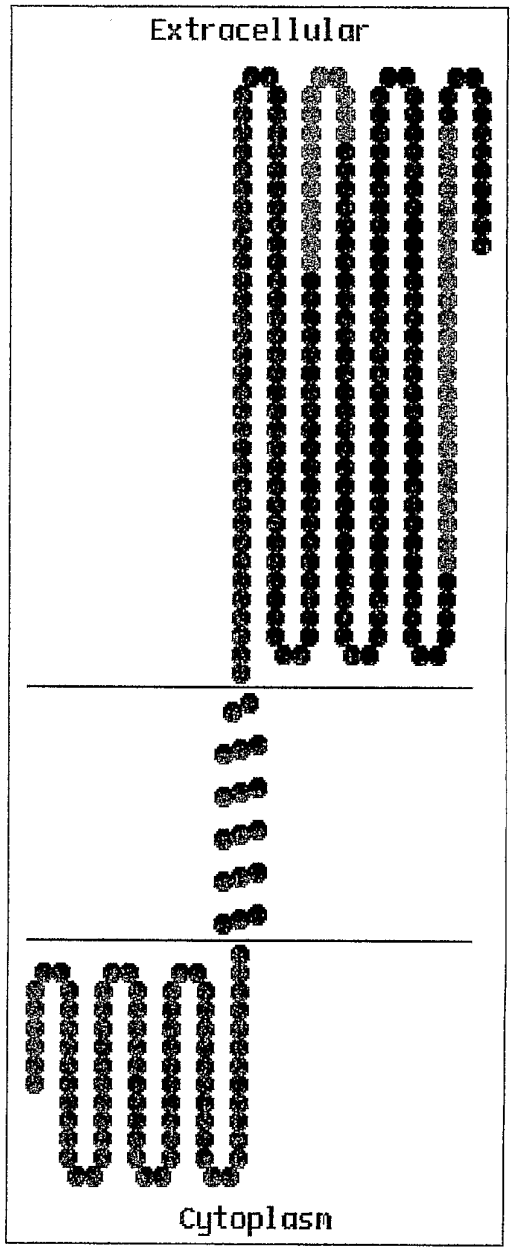
PSORT prediction

Signal Score (-7.5): -0.75 Possible cleavage site: 18
>>> Seems to have no N-terminal signal seq.

count: 1 value: -0.00 threshold: 0.0
INTEGRAL Likelihood = -0.00 Transmembrane 81 - 97 (81 - 97)
PERIPHERAL Likelihood = 2.70 modified ALOM score: 0.50

Rule: cytoplasmic membrane protein

FIG. 76
SPY 405, SPY 1028



GAS	SPY	FACS response
gas406	spy1031	Positive

Features: Membrane

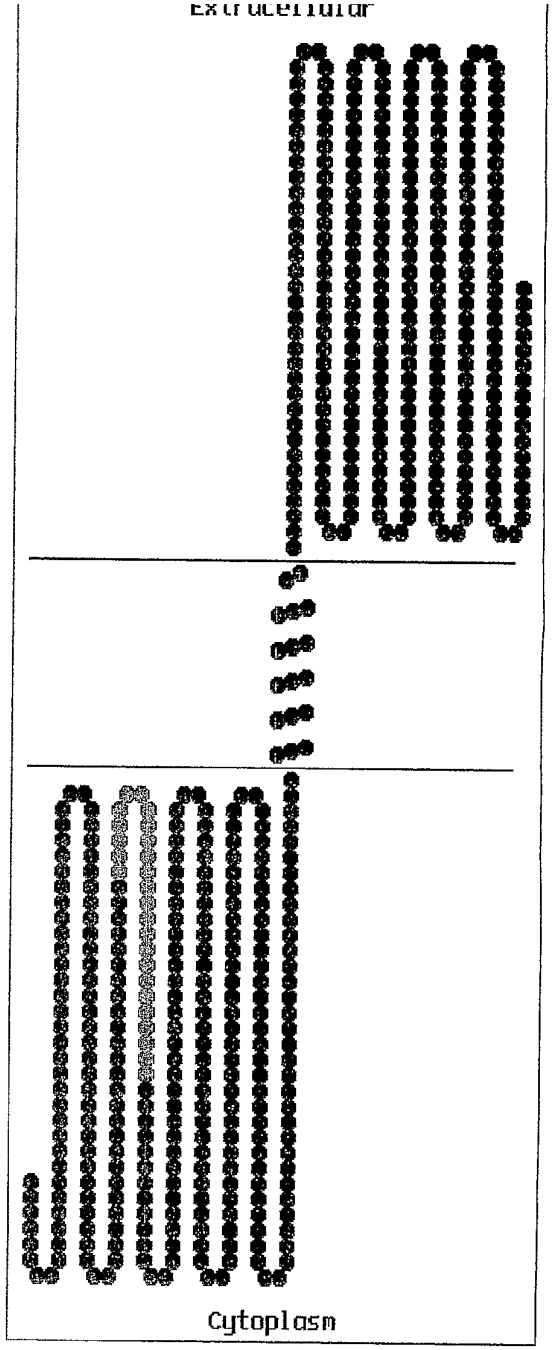
PSORT prediction

Signal Score (-7.5): -7.86 Possible cleavage site: 50
>>> Seems to have no N-terminal signal seq.

count: 1 value: -1.70 threshold: 0.0
INTEGRAL Likelihood = -1.70 Transmembrane 297 - 313 (297 - 315)
PERIPHERAL Likelihood = 4.93 modified ALOM score: 0.84

Rule: cytoplasmic membrane protein

FIG. 77
GAS406, SPY 1031



GAS	SPY	FACS response
		Negative

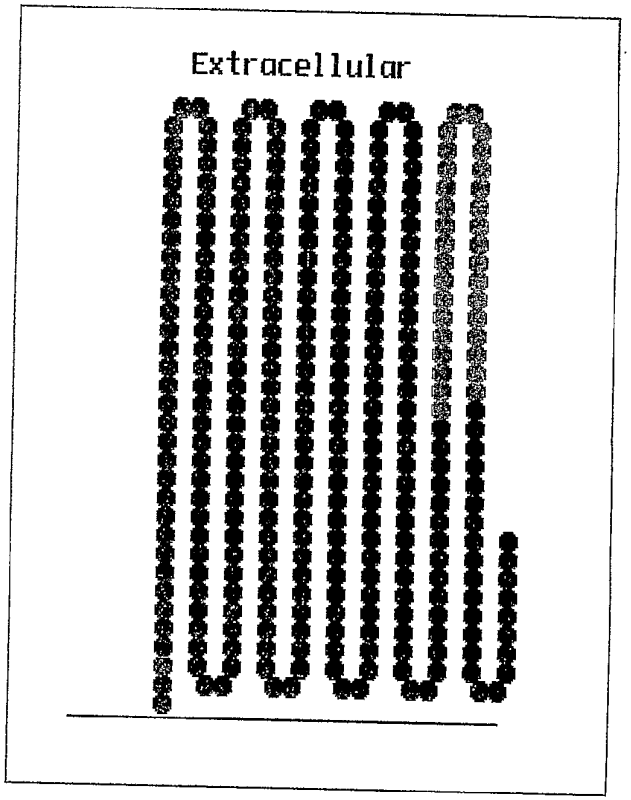
Features: Lipoprotein

PSORT prediction

Possible modific. site: 20 CRend: 3 Sequence Pattern: CTNN

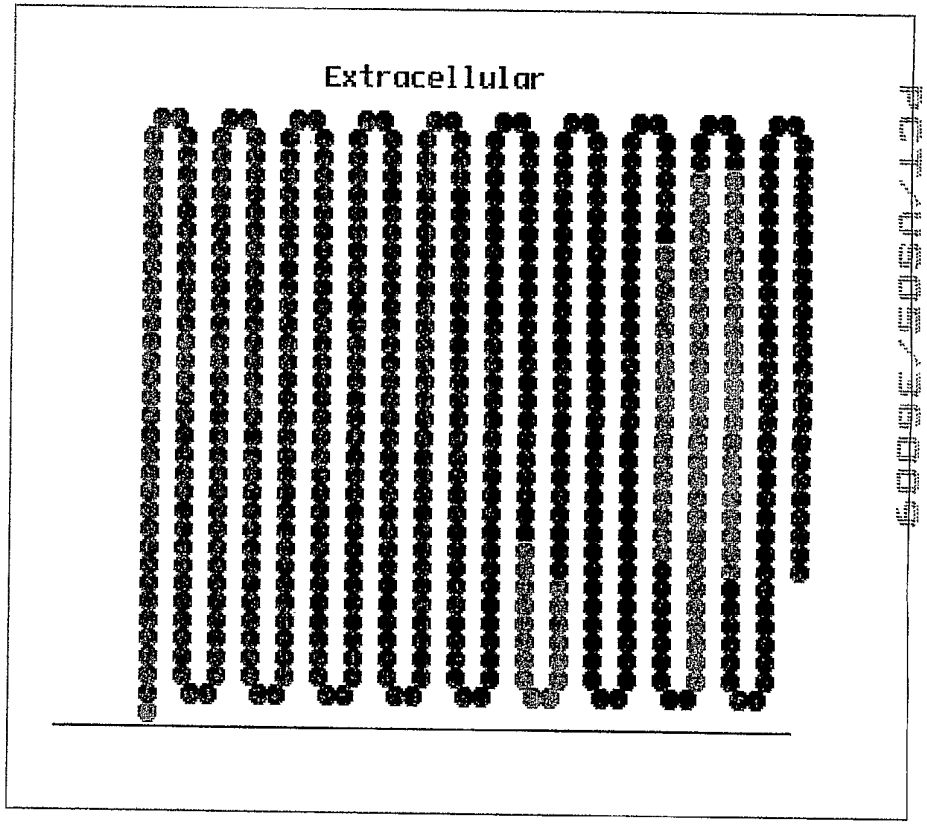
Signal Score (-7.5): -1.5 Possible cleavage site: 17
>>> May be a lipoprotein

FIG. 78
GAS23, SPY 0163



Lipoprotein

[illegible]	[illegible]	FACS response
[illegible]	[illegible]	Negative



Features: Lipoprotein

PSORT prediction

Possible modific. site: 22 CRend: 4
Sequence Pattern: CGNK

Signal Score (-7.5): 0.66 Possible cleavage site: 21
>>> May be a lipoprotein

count: 0 value: 5.04 threshold: 0.0
PERIPHERAL Likelihood = 5.04 modified ALOM score: -1.51

FIG. 79
GAS45; NT01SP0246

GAS	SPY	FACS response
gas49	spy0317	Positive

Features: Lipoprotein

PSORT prediction

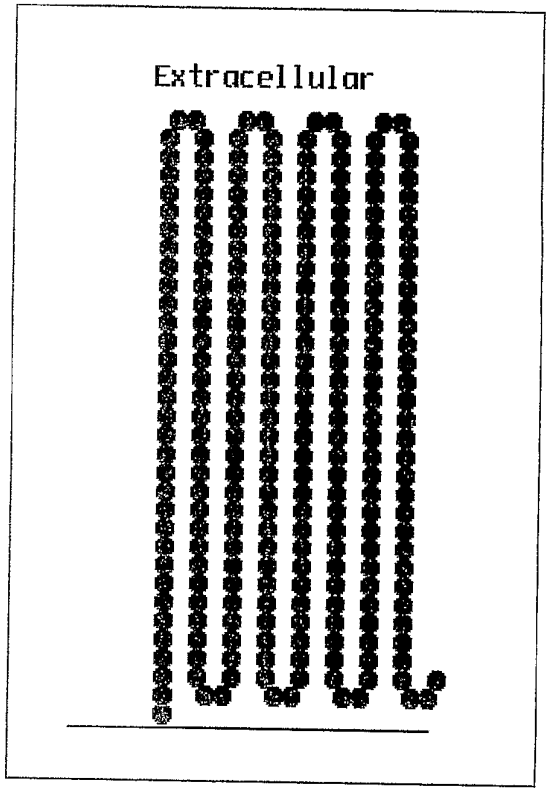
Possible modific. site: 22 CRend: 8
Sequence Pattern: CGSS

Signal Score (-7.5): 1.76 Possible cleavage site: 21
>>> May be a lipoprotein

count: 0 value: 5.46 threshold: 0.0

PERIPHERAL Likelihood = 5.46 modified ALOM score: -1.59 51

FIG. 80
GAS49, SPY 0317



GAS	SPY	FACS response
		Positive

Features: Lipoprotein

PSORT prediction

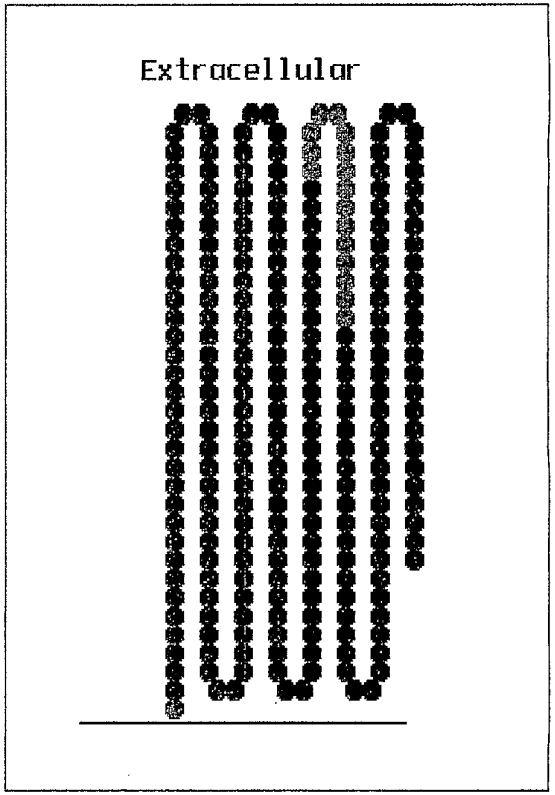
Possible modific. site: 19
CRend: 2
Sequence Pattern: CESV

Signal Score (-7.5): -4.69
Possible cleavage site: 19
>>> May be a lipoprotein

count: 0 value: 6.10 threshold: 0.0

PERIPHERAL Likelihood = 6.10 modified ALOM score: -1.72

FIG. 81
GAS63, SPY 0457



GAS	SPY	FACS response
gas84	spy1274	Positive

Features: Lipoprotein

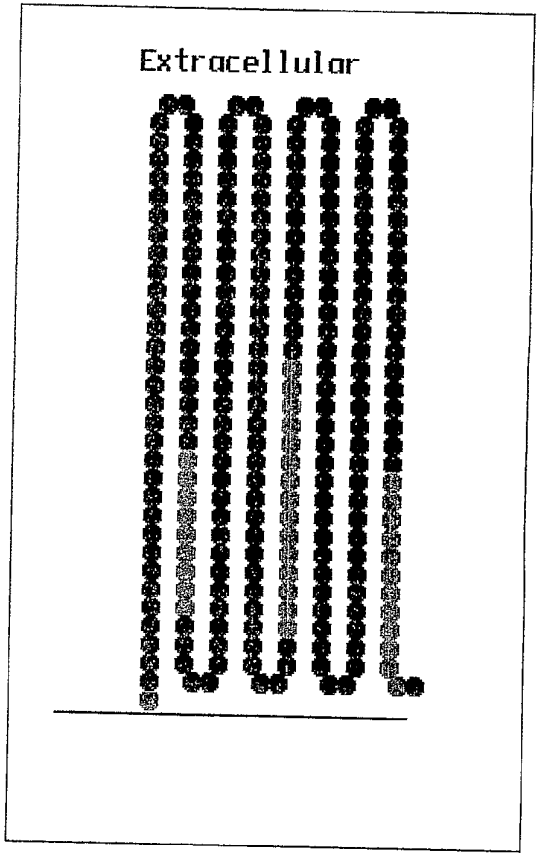
PSORT prediction

Possible modific. site: 21
CRend: 5 Sequence Pattern: CQAT

Signal Score (-7.5): 0.48 Possible cleavage site: 21
>>> May be a lipoprotein

count: 0 value: 10.93 threshold: 0.0
PERIPHERAL Likelihood = 10.93 modified ALOM score: -2.69

FIG. 82
GAS84, SPY 1274



GAS	SPY	FACS response
		Positive

Features: Lipoprotein

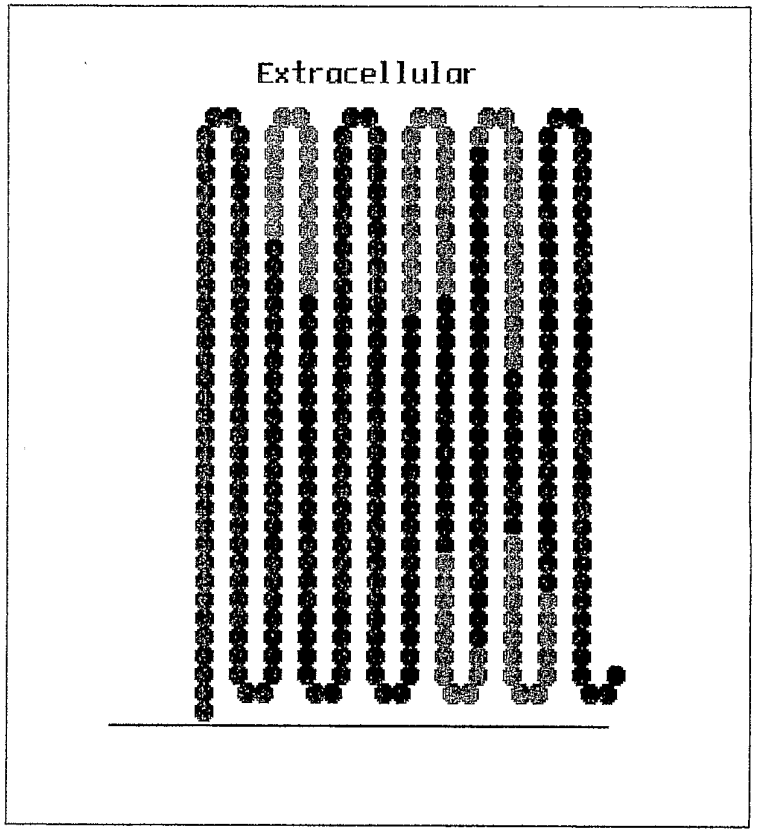
PSORT prediction

Possible modific. site: 23
CRend: 5 Sequence Pattern: CGSG

Signal Score (-7.5): -1 Possible cleavage site: 28
>>> May be a lipoprotein

count: 0 value: 8.43 threshold: 0.0
PERIPHERAL Likelihood = 8.43 modified ALOM score: -2.19

FIG. 83
GAS86, SPY 1294



GAS	SPY	FACS response
		Positive

Features: Lipoprotein

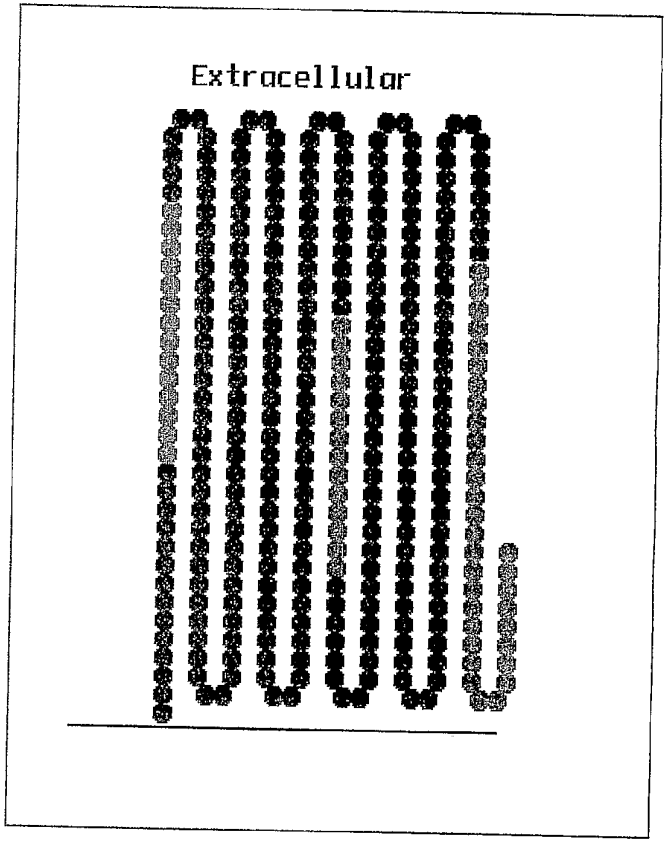
PSORT prediction

Possible modific. site: 22
CRend: 5 Sequence Pattern: CQST

Signal Score (-7.5): 1.6 Possible cleavage site: 21
>>> May be a lipoprotein

count: 0 value: 6.79 threshold: 0.0 PERIPHERAL Likelihood = 6.79
modified ALOM score: -1.86

FIG. 84
GAS89, SPY 1390



GAS	SPY	FACS response
		Positive

Features: Lipoprotein

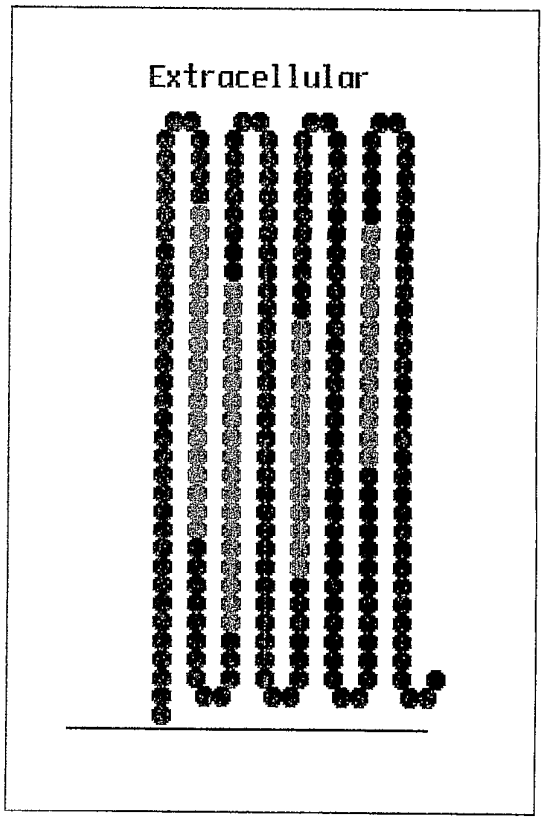
PSORT prediction

Possible modific. site: 21
CRend: 4 Sequence Pattern: CAKV

Signal Score (-7.5): -5 Possible cleavage site: 25
>>> May be a lipoprotein

count: 0 value: 9.81 threshold: 0.0
PERIPHERAL Likelihood = 9.81 modified ALOM score: -2.46

FIG. 85
GAS98, SPY 1882



GAS	SPY	FACS response
gas103	spy2037	Positive

Features: Lipoprotein

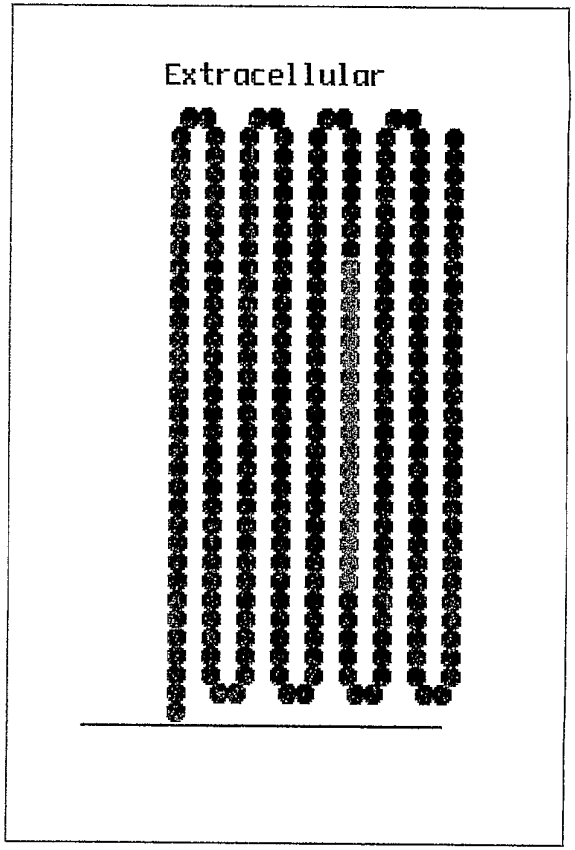
PSORT prediction

Possible modific. site: 22
CRend: 5 Sequence Pattern: CQSS

Signal Score (-7.5): -5.79 Possible cleavage site: 21
>>> May be a lipoprotein

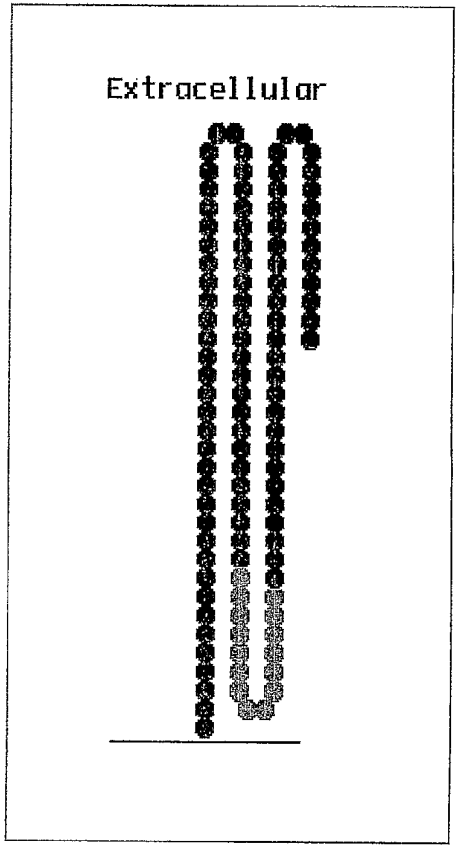
count: 0 value: 6.36 threshold: 0.0 PERIPHERAL Likelihood = 6.36
modified ALOM score: -1.77

FIG. 86
GAS103, SPY 2037



GAS	SPY	FACS response
		Positive

Features: Lipoprotein



PSORT prediction

Possible modific. site: 19
CRend: 5 Sequence Pattern: CSQG

Signal Score (-7.5): -2.5 Possible cleavage site: 19
>>> May be a lipoprotein

count: 0 value: 12.63 threshold: 0.0 PERIPHERAL Likelihood = 12.63

FIG. 87
GAS 108, SPY 0604

		FACS response
		Positive

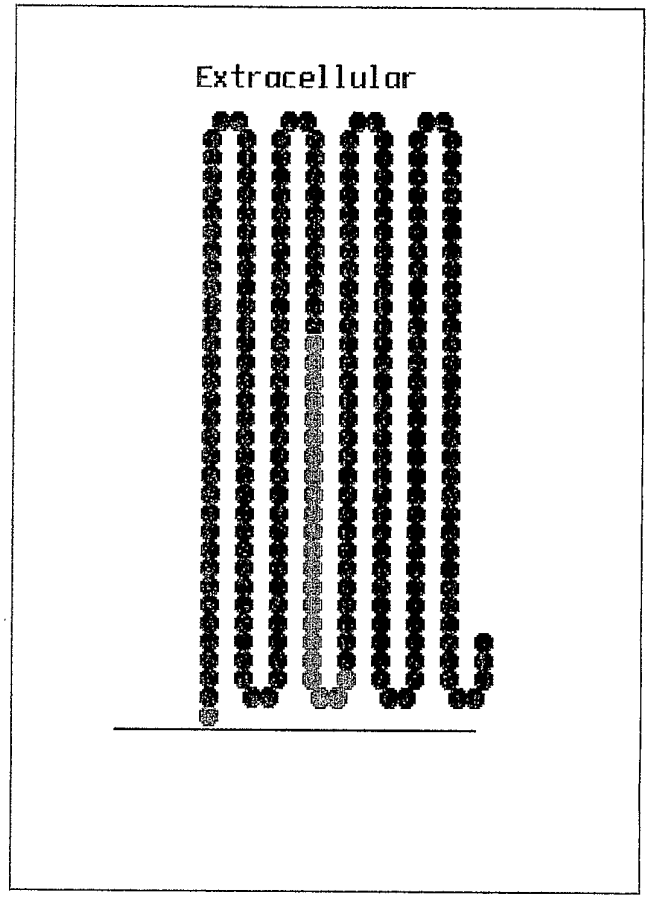
Features: Lipoprotein

PSORT prediction

Possible modific. site: 21 CRend: 4
Sequence Pattern: CSEK

Signal Score (-7.5): -1.64 Possible cleavage site: 21
>>> May be a lipoprotein

FIG. 88
GAS685, SPY 0319



GAS	SPY	FACS response
		Positive

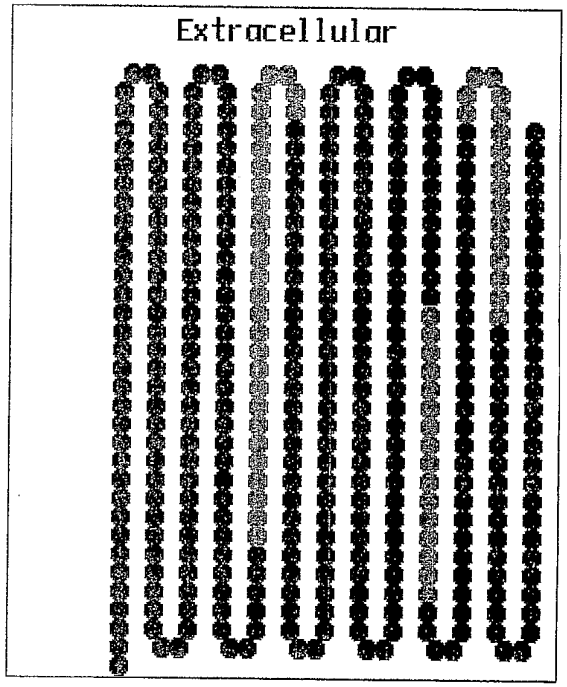
Features: Outside

PSORT prediction

Signal Score (-7.5): 0.759999 Possible cleavage site: 33
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 5.57 threshold: 0.0 PERIPHERAL
Likelihood = 5.57 modified ALOM score: -1.61

FIG. 89
GAS24, SPY 0165



Outside

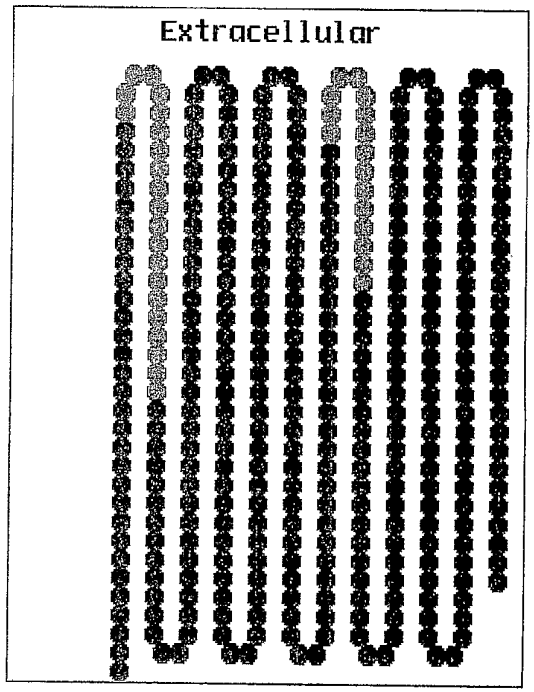
GAS	SPY	FACS response
		Positive

Features: Outside

PSORT prediction

Signal Score (-7.5): -0.200001 Possible cleavage site: 17
>>> Seems to have a cleavable N-term signal seq.

FIG. 90
GAS5, SPY 0019



GAS	SPY	FACS response
		Negative

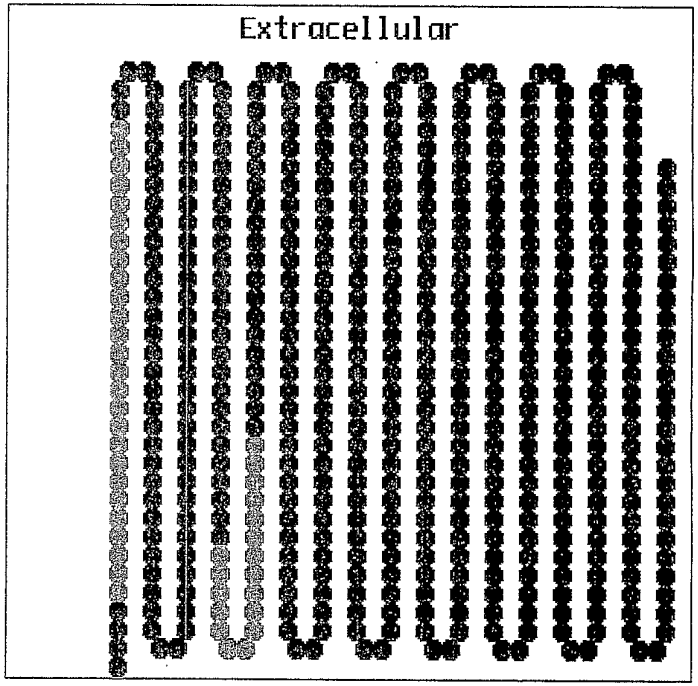
Features: Outside

PSORT prediction

Signal Score (-7.5): -1.69 Possible cleavage site: 31
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 5.62 threshold: 0.0 PERIPHERAL
Likelihood = 5.62 modified ALOM score: -1.62

FIG. 91
GAS25, SPY 0167



GAS	SPY	FACS response
		Positive

Features: Outside

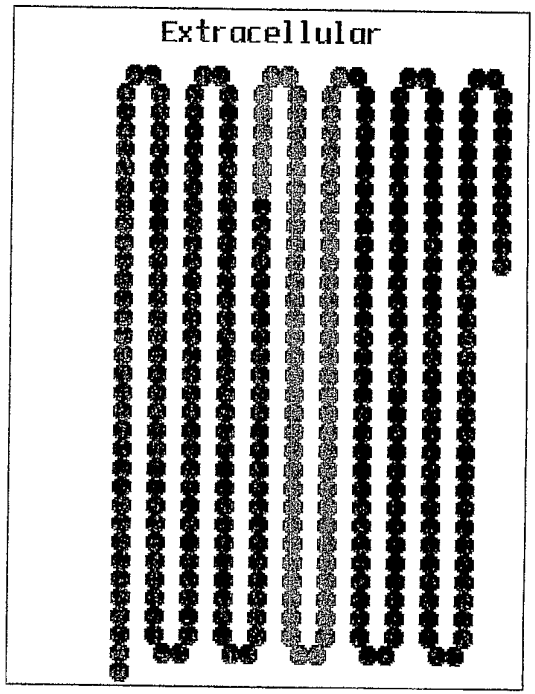
PSORT prediction

Signal Score (-7.5): 2.56
Possible cleavage site: 25
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 4.83 threshold: 0.0

PERIPHERAL Likelihood = 4.83 modified ALOM score: -1.47

FIG. 92
GAS64, SPY 0469



GAS	SPY	FACS response
		NOT CLONED

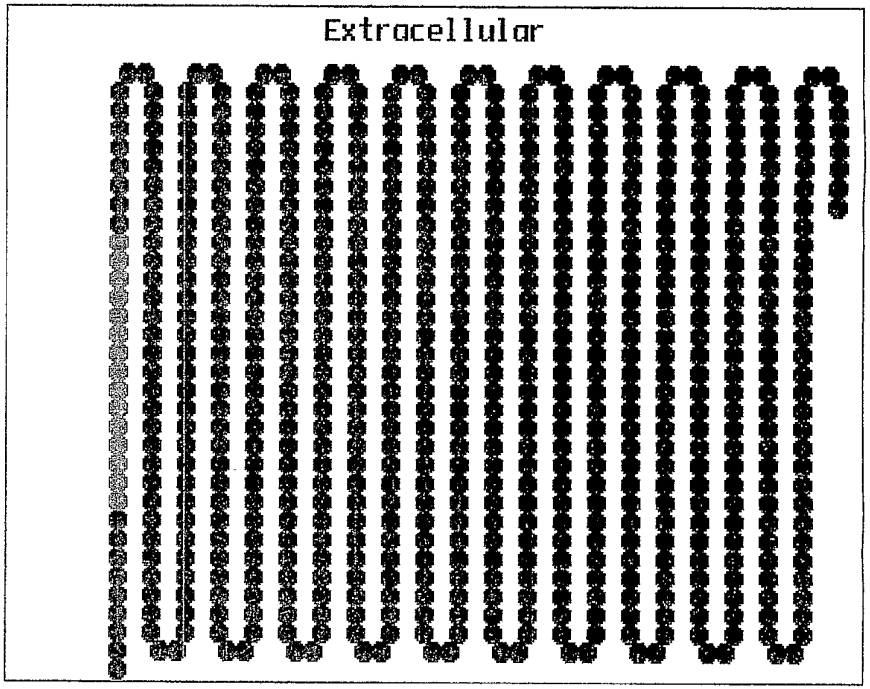
Features: Outside

PSORT prediction

Signal Score (-7.5): 2.51 Possible cleavage site: 30
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 4.14 threshold: 0.0
PERIPHERAL Likelihood = 4.14 modified ALOM score: -1.33

FIG. 93
GAS87, SPY 1302



GAS	SPY	FACS response
		Positive

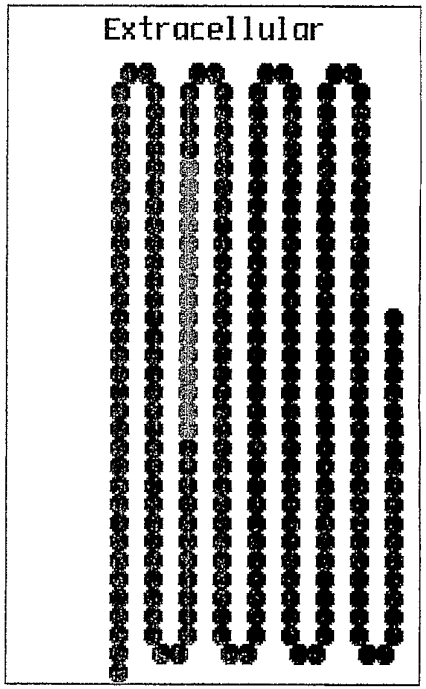
Features: Outside

PSORT prediction

Signal Score (-7.5): 2.06 Possible cleavage site: 32
>>> Seems to have a cleavable N-term signal seq.

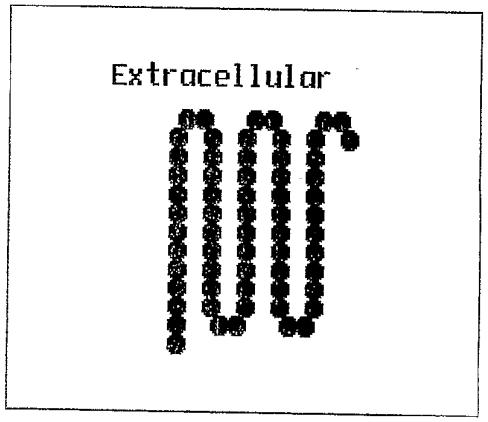
count: 0 value: 15.86 threshold: 0.0
PERIPHERAL Likelihood = 15.86 modified ALOM score: -3.67

FIG. 94
GAS102, SPY 2016



GAS	SPY	FACS response
gas362	spy1461	Positive

Features: Outside



PSORT prediction

Signal Score (-7.5): 1.08 Possible cleavage site: 25
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 8.17 threshold: 0.0
PERIPHERAL Likelihood = 8.17 modified ALOM score: -2.13

FIG. 95
GAS362, SPY 1461

GAS	SPY	FACS response
		Positive

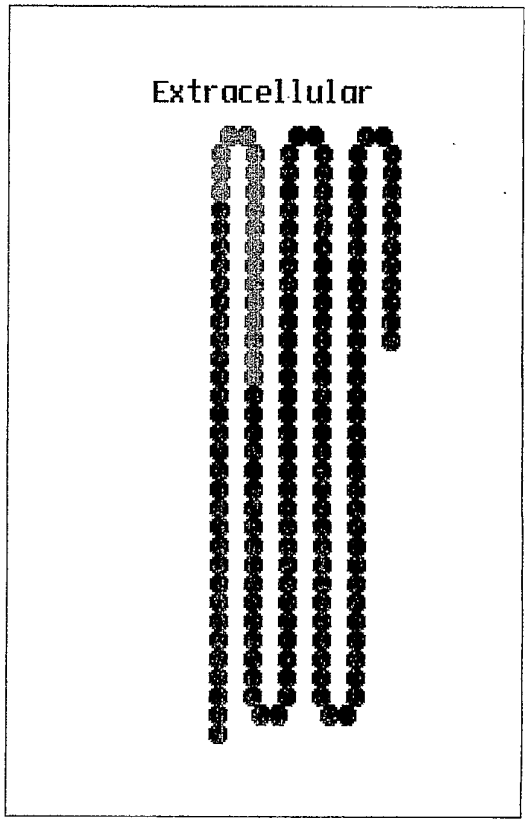
Features: Outside (membrane?)

PSORT prediction

Signal Score (-7.5): -1.6 Possible cleavage site: 43
>>> Seems to have a cleavable N-term signal seq.

count: 0 value: 5.73 threshold: 0.0
PERIPHERAL Likelihood = 5.73 modified ALOM score: -1.65

FIG. 96
GAS382, SPY 1842



[illegible]	[illegible]	FACS response	
[illegible]	[illegible]	NOT CLONED	putative signal peptidase I

Features: ?

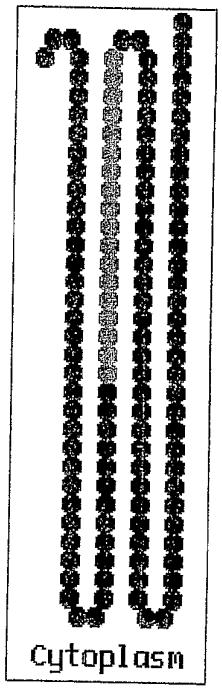
PSORT prediction

Signal Score (-7.5): -3 Possible cleavage site: 14
>>> Seems to have no N-terminal signal seq.

count: 0 value: 9.02 threshold: 0.0 PERIPHERAL Likelihood = 9.02
modified ALOM score: -2.30

Rule: cytoplasmic protein

FIG. 97
SPY 0127



Cytoplasmic

GAS	SPY	FACS response	
□s	s□□□□□□	NOT CLONED	hypothetical protein, phage associated

Features: ?

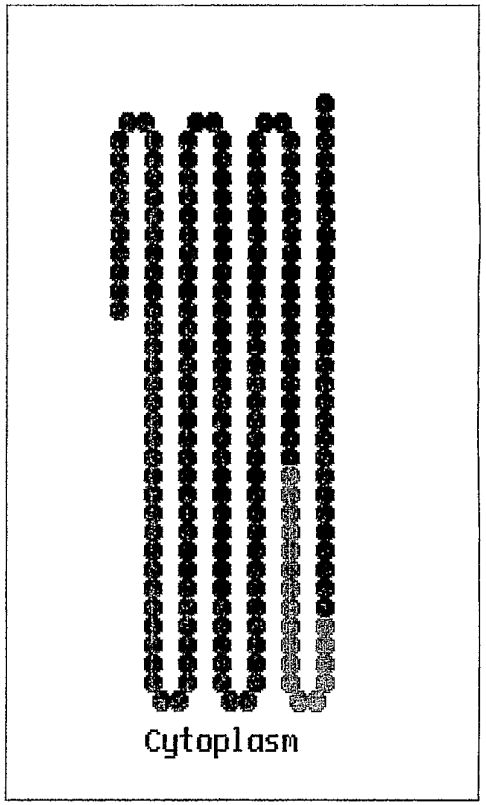
PSORT prediction

Signal Score (-7.5): -4.21 Possible cleavage site: 60
>>> Seems to have no N-terminal signal seq.

count: 0 value: 5.62 threshold: 0.0 PERIPHERAL Likelihood = 5.62
modified ALOM score: -1.62

Rule: cytoplasmic protein

FIG. 98
SPY 0686



□	Spy0792	NOT CLONED

conserved hypothetical protein, possibly involved in cell wall localization and side chain formation of polysacch.

Features: ?

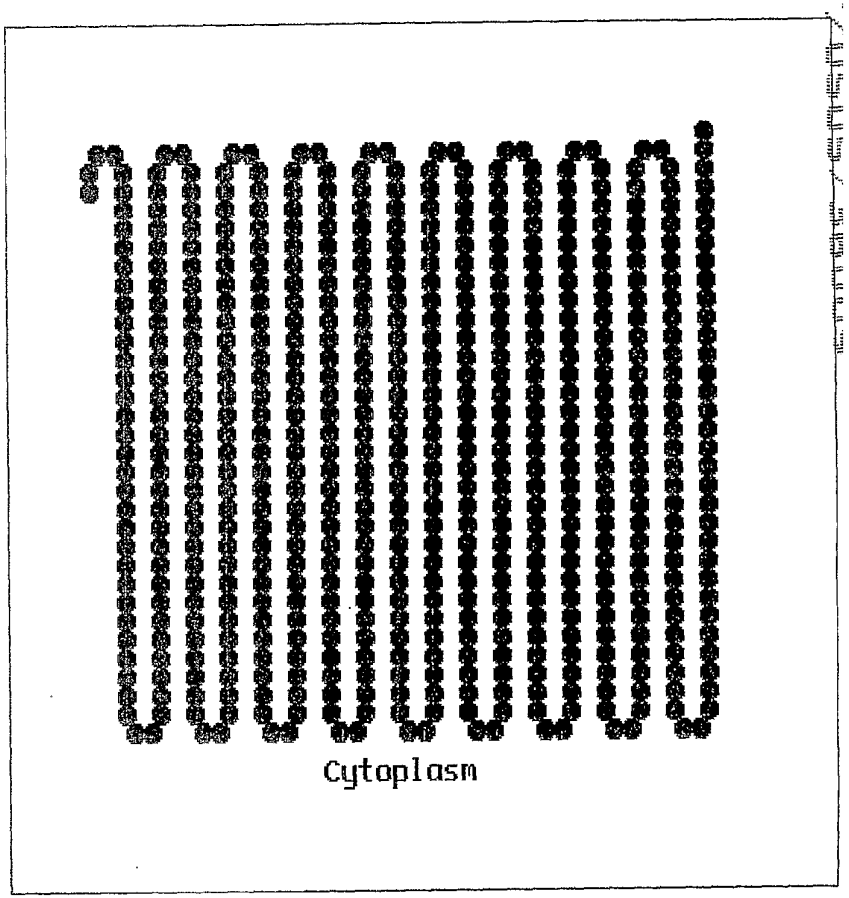
PSORT prediction

Signal Score (-7.5): -5.73 Possible cleavage site: 32
>>> Seems to have no N-terminal signal seq.

count: 0 value: 2.97 threshold: 0.0 PERIPHERAL Likelihood = 2.97
modified ALOM score: -1.09

Rule: cytoplasmic protein

FIG. 99
SPY 0792



[illegible]	[illegible]	FACS response	
[illegible]	[illegible]	NOT CLONED	putative dihydrolipoamide S-acetyltransferase

Features: ?

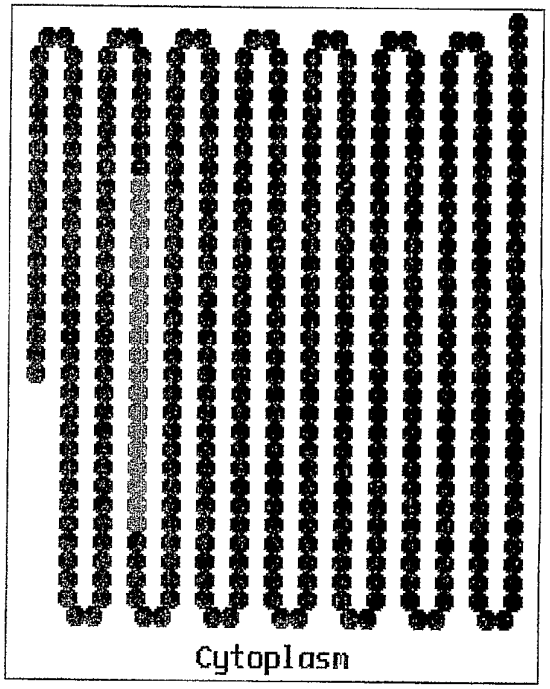
PSORT prediction

Signal Score (-7.5): -7.86 Possible cleavage site: 50
>>> Seems to have no N-terminal signal seq.

count: 0 value: 0.32 threshold: 0.0 PERIPHERAL Likelihood = 0.32
modified ALOM score: -0.56

Rule: cytoplasmic protein

FIG. 100
SPY 1029



GAS	SPY	FACS response	
□S	SP□□□□	NOT CLONED	hypothetical protein

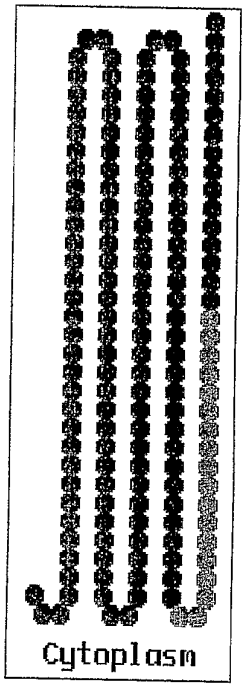
Features: ?

PSORT prediction

Signal Score (-7.5): -4.75 Possible cleavage site: 43
>>> Seems to have no N-terminal signal seq.
count: 0 value: 3.13 threshold: 0.0 PERIPHERAL Likelihood = 3.13
modified ALOM score: -1.13

Rule: cytoplasmic protein

FIG. 101
SPY 1260



[illegible]	[illegible]	FACS response	
[illegible]	[illegible]	NOT CLONED	conserved protein - function unknown

Features: ?

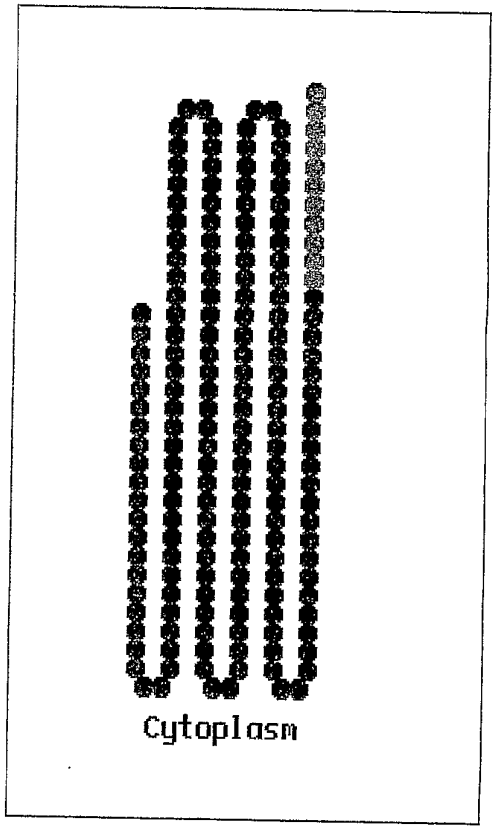
PSORT prediction

Signal Score (-7.5): -7.65 Possible cleavage site: 16
>>> Seems to have no N-terminal signal seq.

count: 0 value: 9.81 threshold: 0.0 PERIPHERAL Likelihood = 9.81
modified ALOM score: -2.46

Rule: cytoplasmic protein

FIG. 102
SPY 1613



[illegible]	[illegible]	FACS response	
[illegible]	[illegible]	NOT CLONED	putative thioredoxin

Features: ?

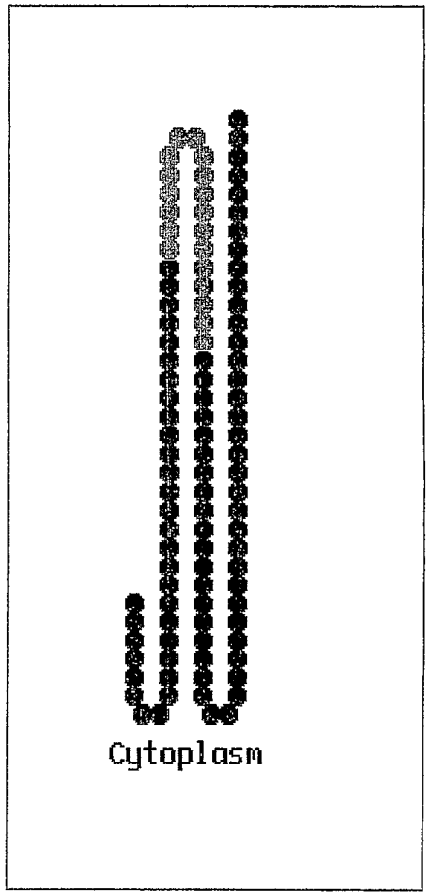
PSORT prediction

Signal Score (-7.5): -5.28 Possible cleavage site: 34
>>> Seems to have no N-terminal signal seq.

count: 0 value: 6.36 threshold: 0.0 PERIPHERAL Likelihood = 6.36
modified ALOM score: -1.77

Rule: cytoplasmic protein

FIG. 103
SPY 1835



[illegible]	[illegible]	FACS response
[illegible]	[illegible]	NOT CLONED

hypothetical protein

Features: ?

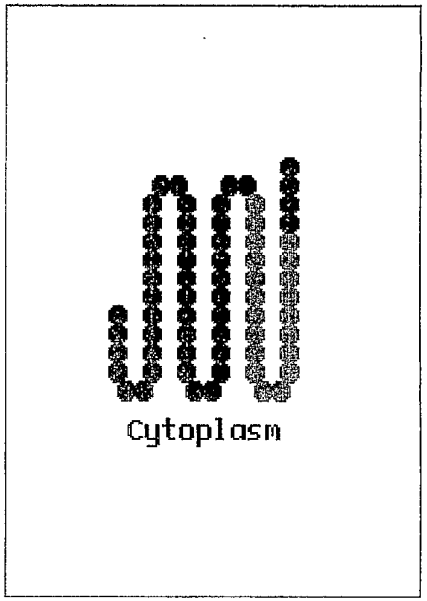
PSORT prediction

Signal Score (-7.5): -6.52 Possible cleavage site: 41
>>> Seems to have no N-terminal signal seq.

count: 0 value: 14.22 threshold: 0.0 PERIPHERAL Likelihood = 14.22
modified ALOM score: -3.34

Rule: cytoplasmic protein

FIG. 104
SPY 2005



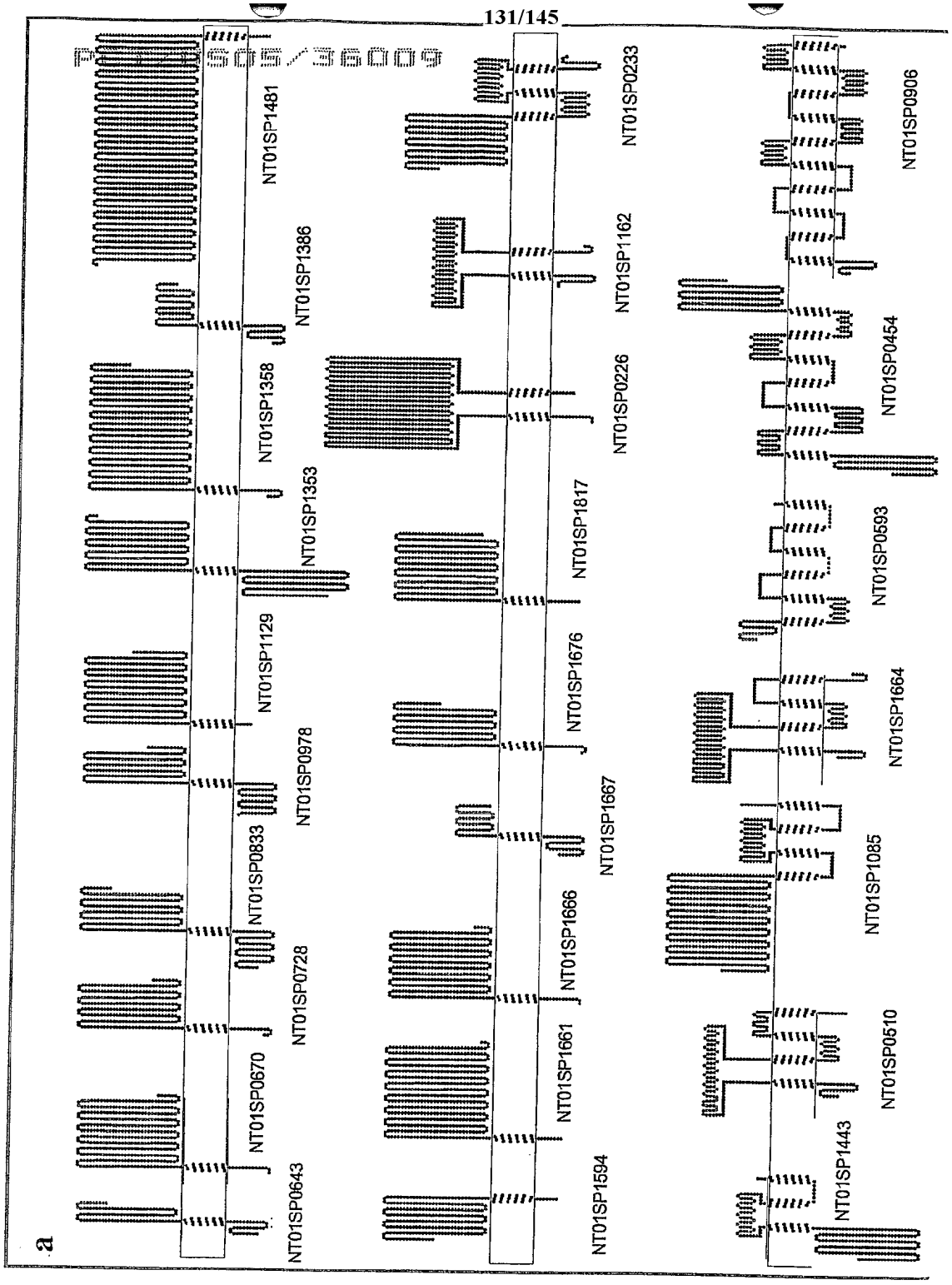

a
NT01SP0643
NT01SP0670
NT01SP0728
NT01SP0833
NT01SP0978
NT01SP1129
NT01SP1353
NT01SP1358
NT01SP1386
NT01SP1481
NT01SP1594
NT01SP1661
NT01SP1666
NT01SP1667
NT01SP1676
NT01SP1817
NT01SP0226
NT01SP1162
NT01SP0233
NT01SP1443
NT01SP0510
NT01SP1085
NT01SP1664
NT01SP0593
NT01SP0454
NT01SP0906

FIG. 10SA

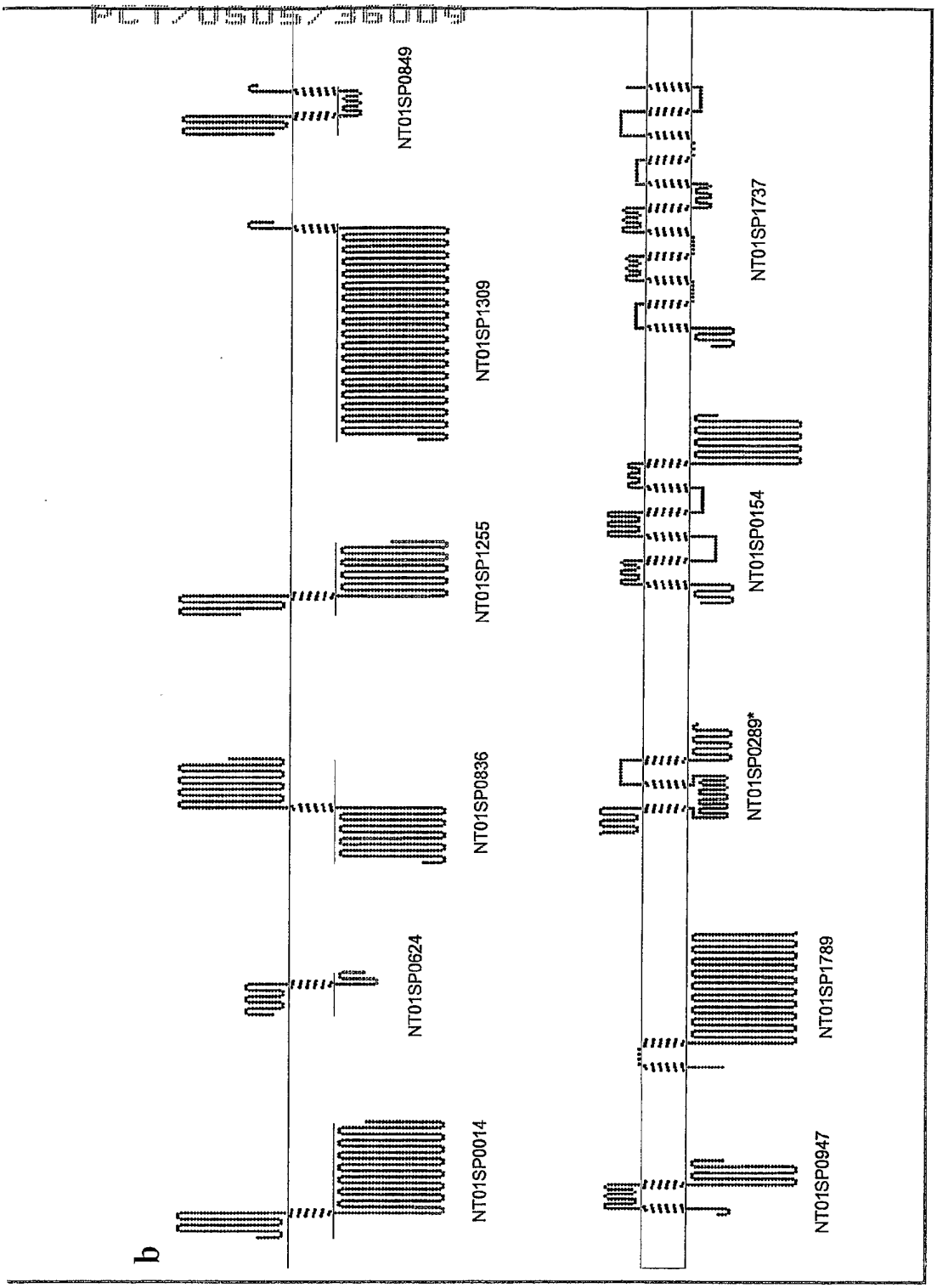
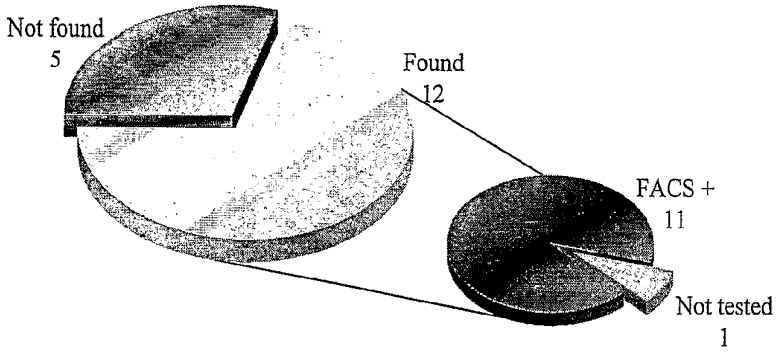
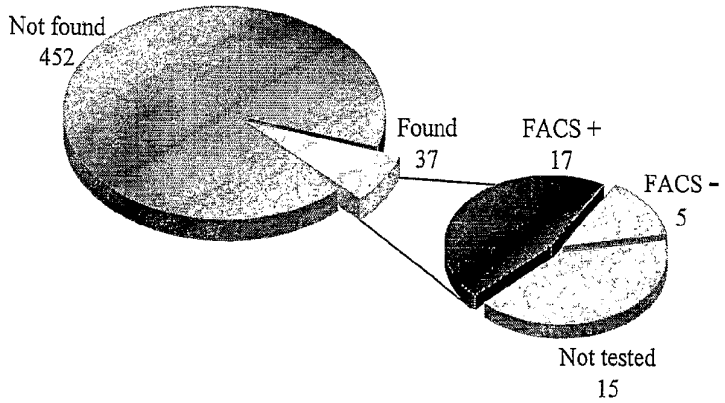

b
NT01SP0014
NT01SP0624
NT01SP0836
NT01SP1255
NT01SP1309
NT01SP0849
NT01SP0947
NT01SP1789
NT01SP0289*
NT01SP0154
NT01SP1737

FIG. 105B

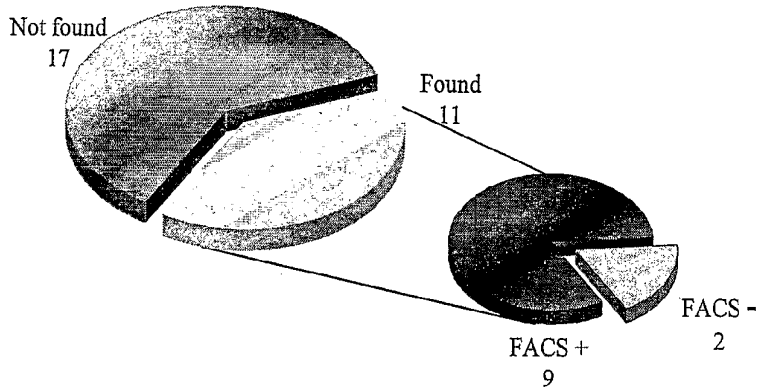
LPXTG Proteins
(17 predicted by in silico analysis)



Membrane Proteins
(489 predicted by in silico analysis)



Lipoproteins
(28 predicted by in silico analysis)



Secreted Proteins
(67 predicted by in silico analysis)

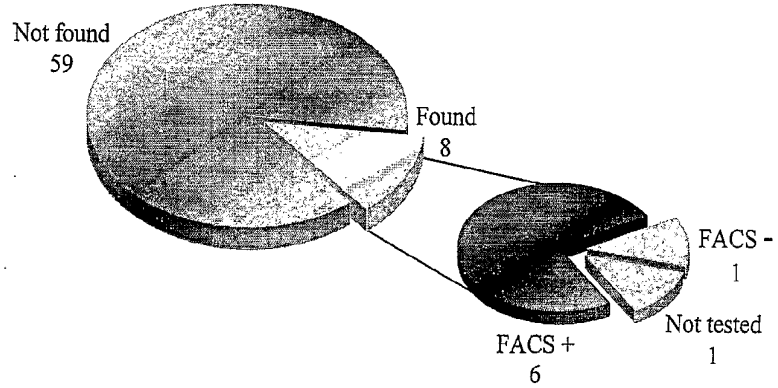


FIG. 106

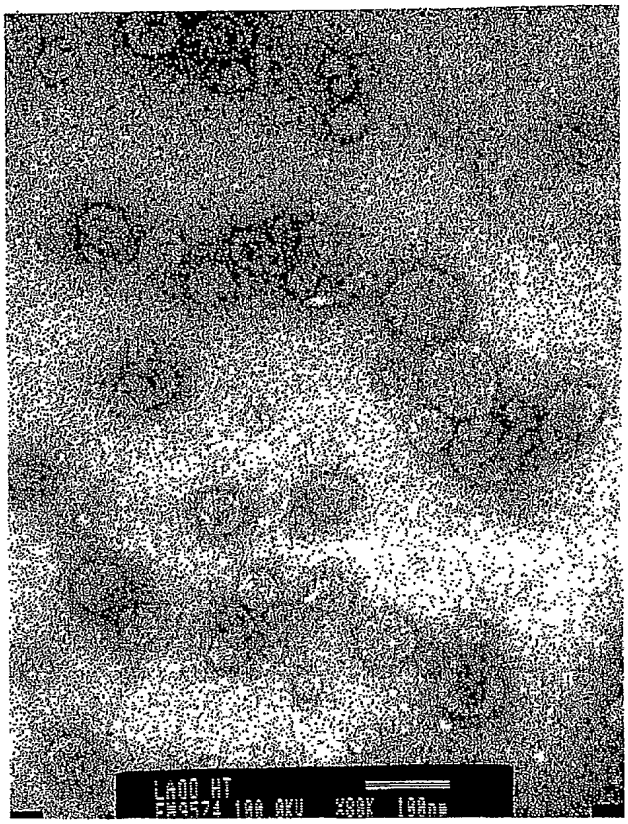


FIG. 107

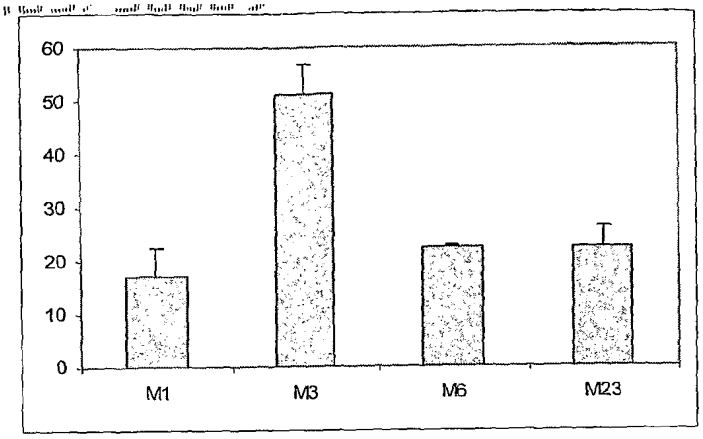
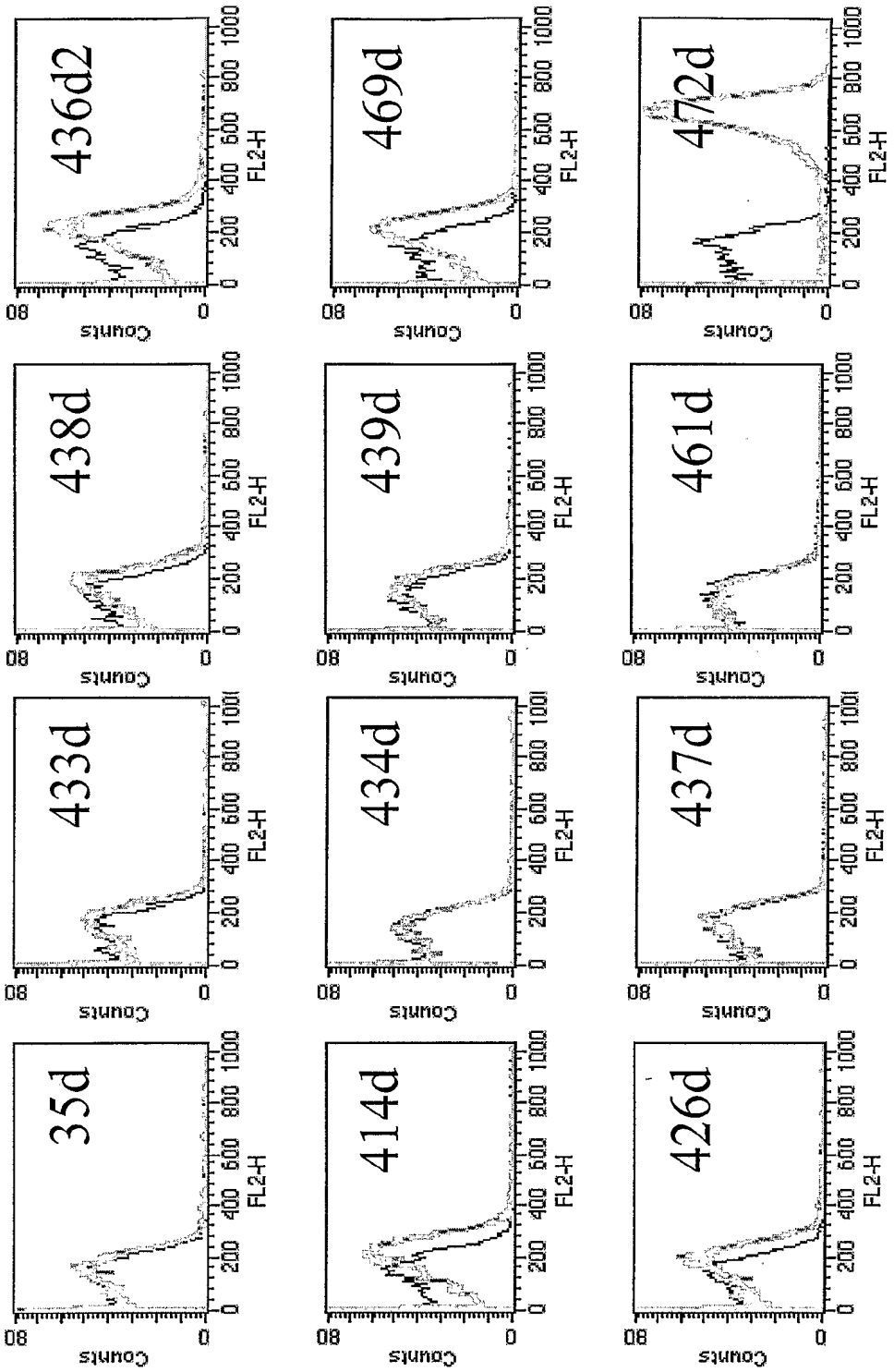


FIG. 108

FIG. 109A

DSM 2071 M23



DSM 2071 M23

FIG. 109B

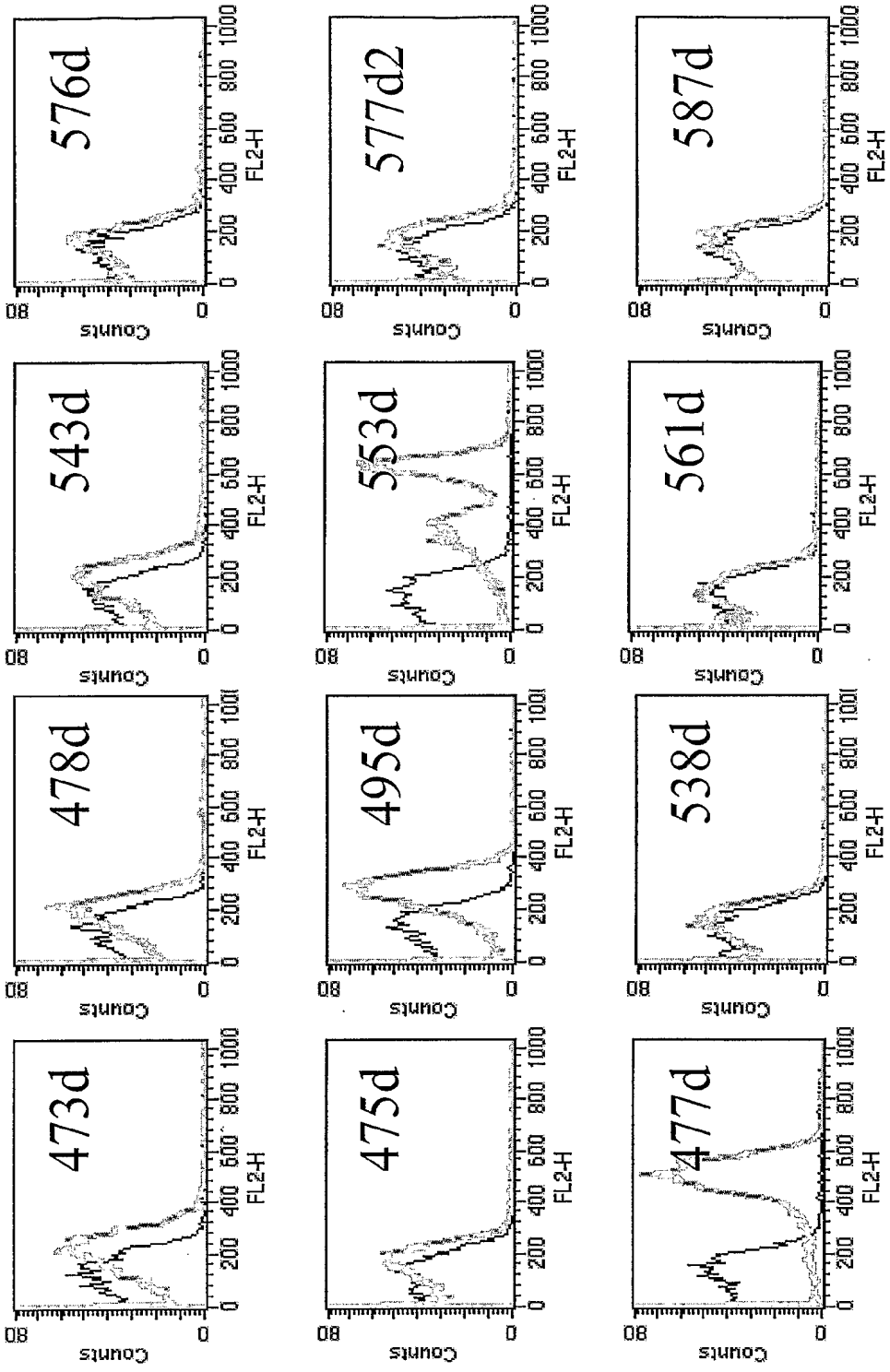


FIG. 109C

DSM 2071 M23

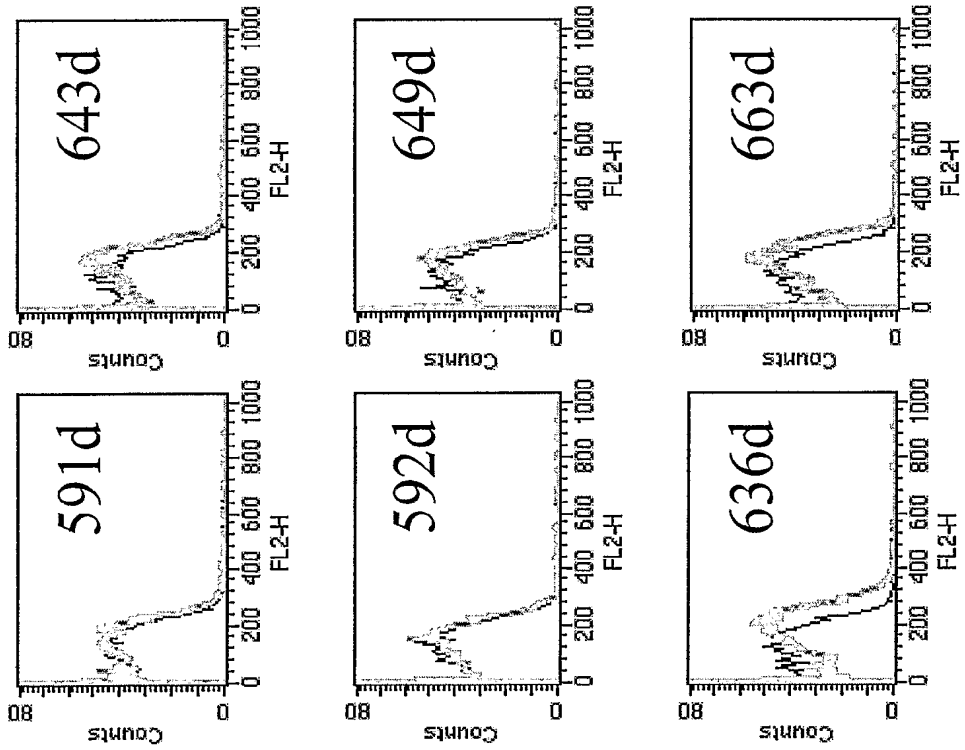


FIG. 110A

3348 M1

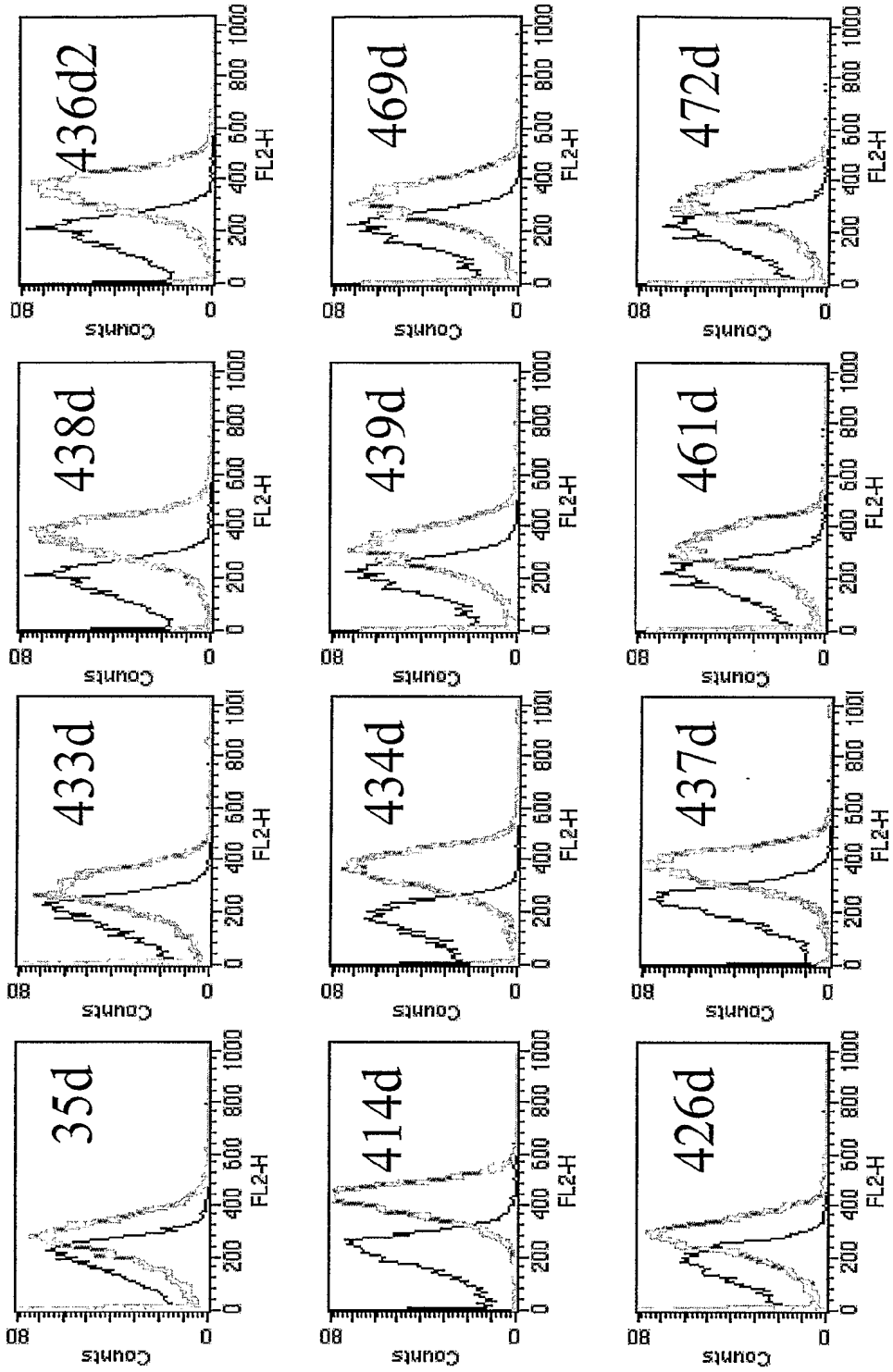


FIG. 110B

3348 M1

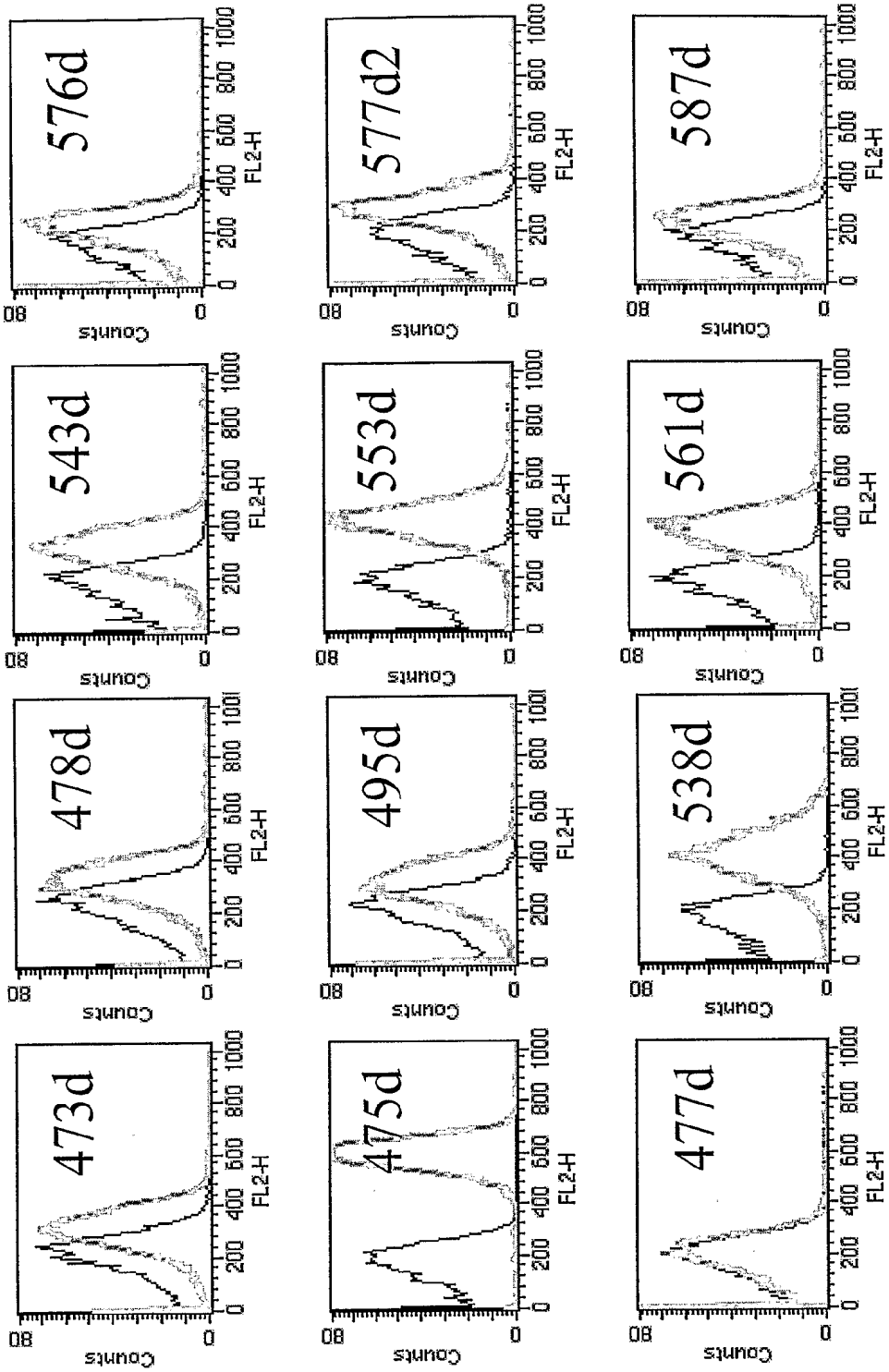


FIG. 110C

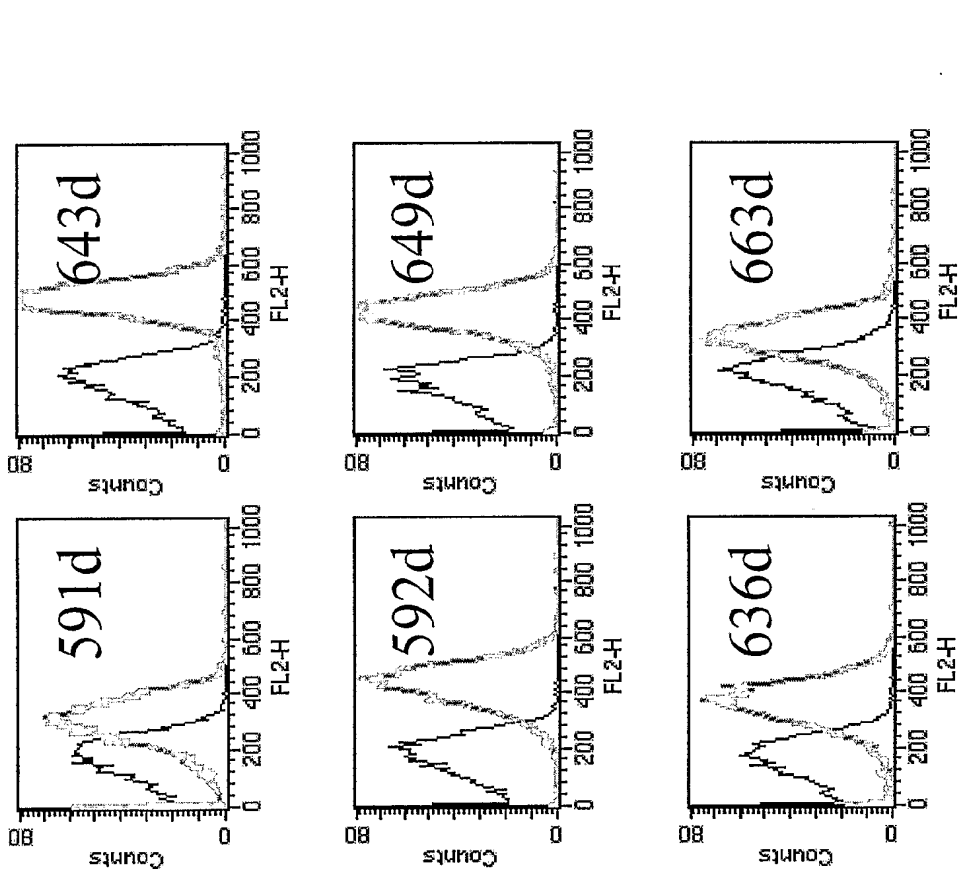

3348 M1
643d
591d
649d
592d
663d
636d
Counts
FL2-H
0
200
400
600
800
1000
80

FIG. 111A

2728 EM5 M12

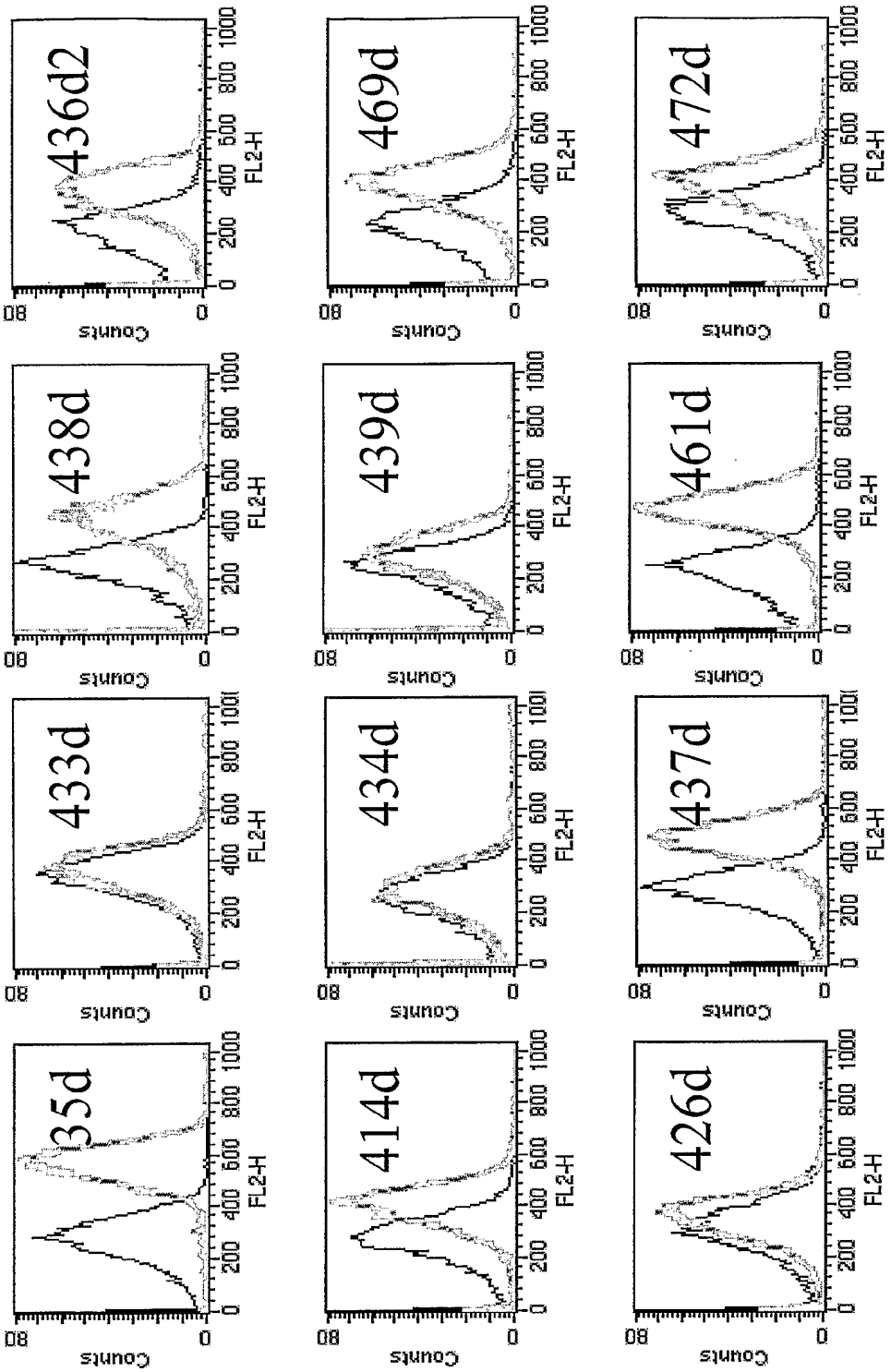


FIG. 111B

2728 EM5 M12

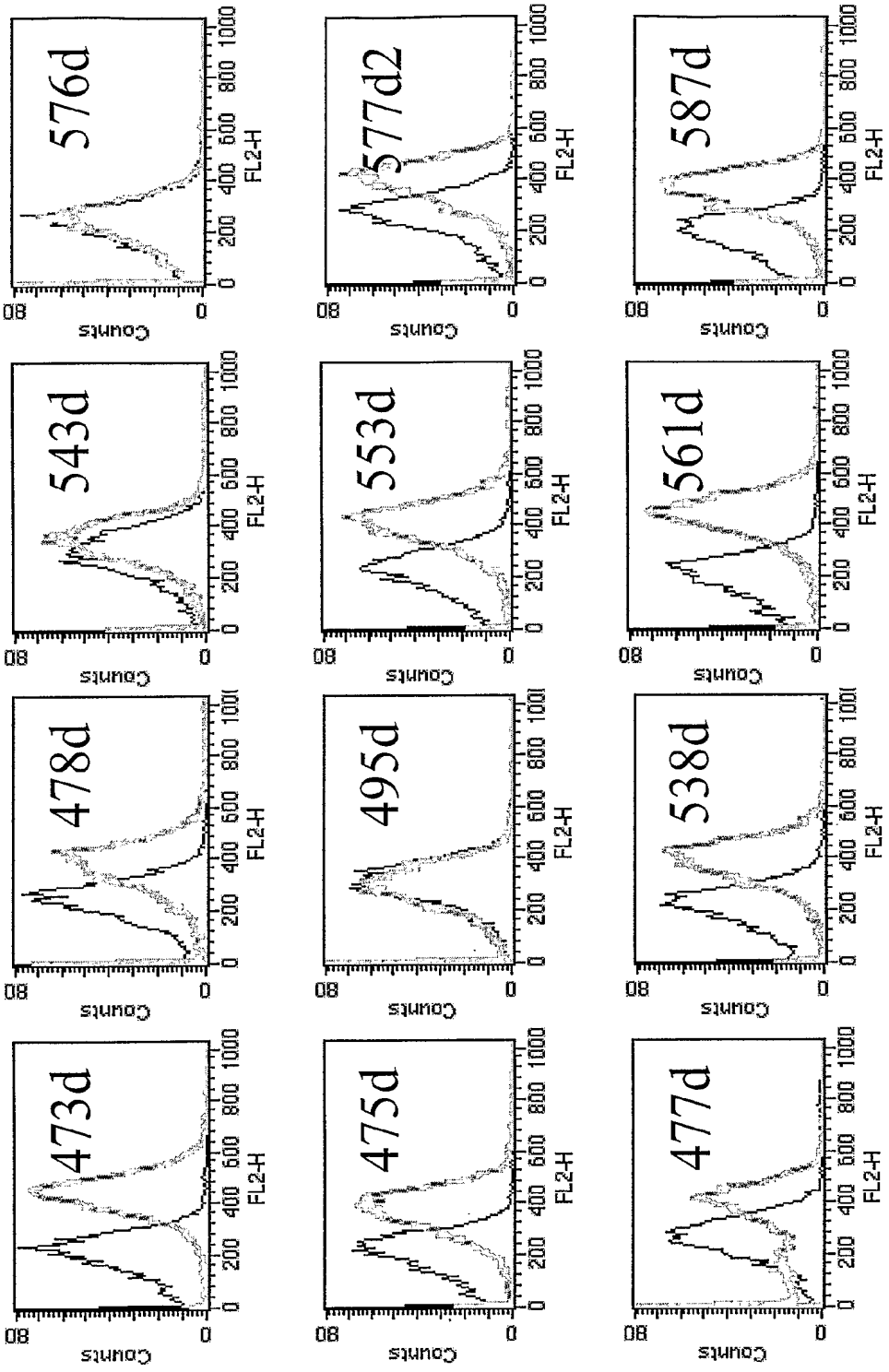
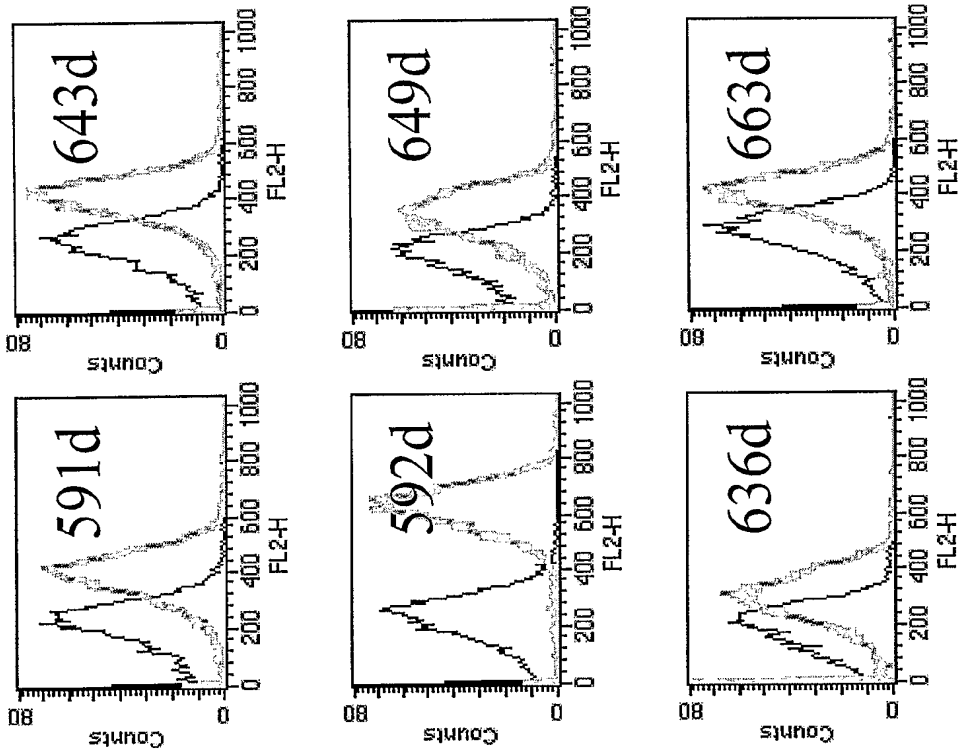


FIG. 111C

2728 EM5 M12



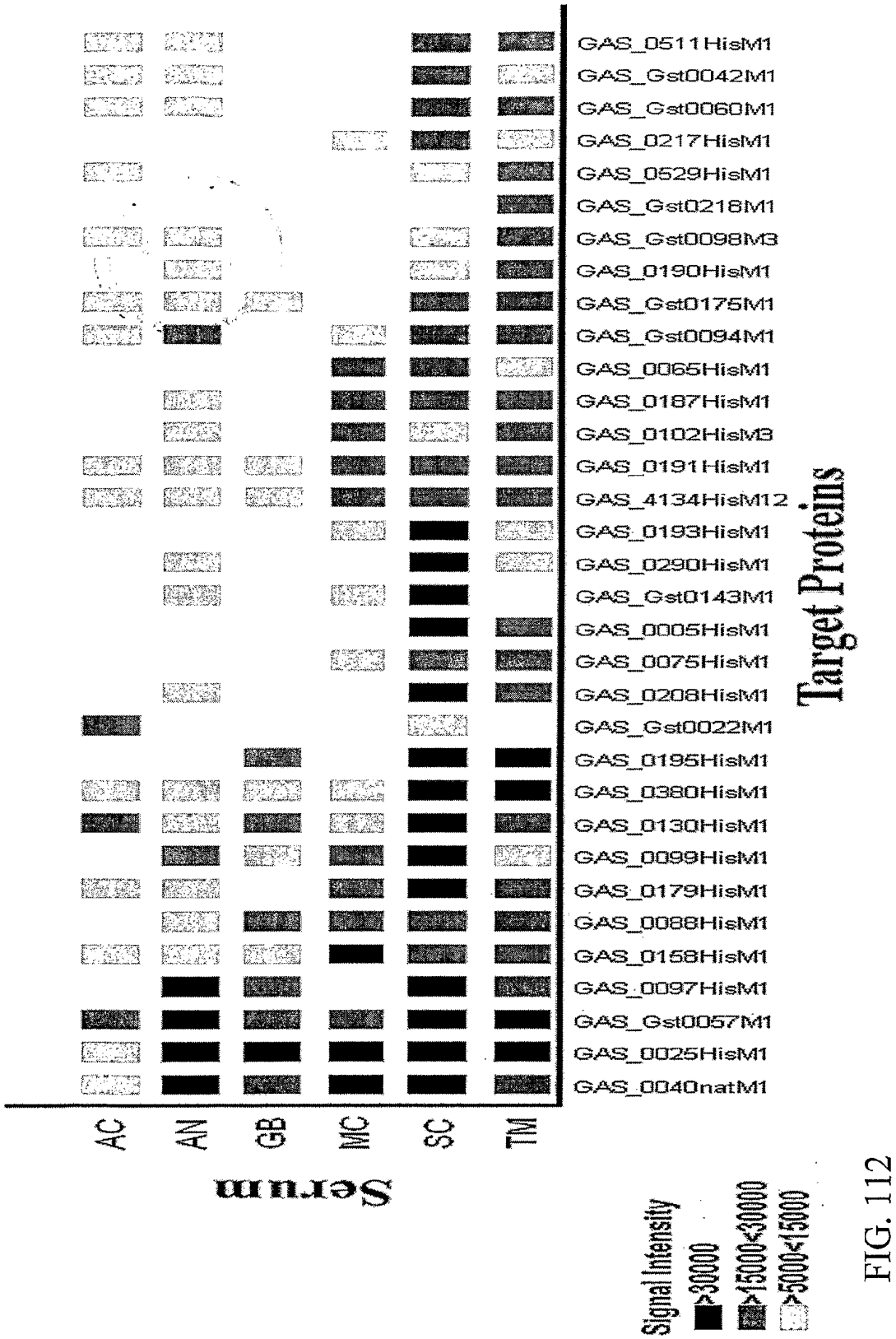

GAS_0511HisM1
GAS_Gst0042M1
GAS_Gst0060M1
GAS_0217HisM1
GAS_0529HisM1
GAS_Gst0218M1
GAS_Gst0098M3
GAS_0190HisM1
GAS_Gst0175M1
GAS_Gst0094M1
GAS_0065HisM1
GAS_0187HisM1
GAS_0102HisM3
GAS_0191HisM1
GAS_4134HisM12
GAS_0193HisM1
GAS_0290HisM1
GAS_Gst0143M1
GAS_0005HisM1
GAS_0075HisM1
GAS_0208HisM1
GAS_Gst0022M1
GAS_0195HisM1
GAS_0380HisM1
GAS_0130HisM1
GAS_0099HisM1
GAS_0179HisM1
GAS_0088HisM1
GAS_0158HisM1
GAS_0097HisM1
GAS_Gst0057M1
GAS_0025HisM1
GAS_0040natM1
Target Proteins
AC
AN
GB
MC
SC
TM
Serum
Signal Intensity
>30000
>15000<30000
>5000<15000

FIG. 112

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(74) Agents: **HEMMENDINGER, Lisa, M.** et al.; Banner & Witcoff, Ltd., 1001 G Street, N.W.., 11th Floor, Washington, D.C. 20001-4597 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/036009

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2006	20. 06. 2006
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Mauhin, V

6

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/078907 A (INTERCELL AG; MEINKE, ANDREAS; NAGY, ESZTER; WINKLER, BIRGIT; GELBMANN) 16 September 2004 (2004-09-16) page 9, line 49 - page 10, line 21 page 12, line 15 - line 16 page 14, line 3 - line 4 page 22, line 32 - line 39 page 24, line 4 - line 5 page 31, line 31 - page 32, line 22 page 35, line 50 - page 37, line 28 page 39, line 16 - line 30 page 40, line 8 - line 22 spy0019 of SEQ. ID. No. 152 is 100% identical to present GAS5 (SEQ. ID. No. 2). Specific epitopes are disclosed. The encoding DNA of SEQ. ID. No. 2 is 100% identical to present GAS5 (SEQ. ID. No. 651) figure 4a; example 5; tables 1,3	1-40
X	DATABASE UniProt [Online] 1 October 2002 (2002-10-01), XP002367841 retrieved from EBI Database accession no. Q8P318 the whole document	1,24
X	-& NAKAGAWA ICHIRO ET AL: "Genome sequence of an M3 strain of Streptococcus pyogenes reveals a large-scale genomic rearrangement in invasive strains and new insights into phage evolution." GENOME RESEARCH, vol. 13, no. 6a, June 2003 (2003-06), pages 1042-1055, XP002367632 ISSN: 1088-9051 protein SPS0015 SPYM3_0014 is 99,497% identical to present GAS5 over the whole sequence.	1,24
X	DATABASE UniProt [Online] 5 July 2004 (2004-07-05), XP002367842 retrieved from EBI Database accession no. Q7CNQ7 the whole document	1,24

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	-& SMOOT J C ET AL: "Genome sequence and comparative microarray analysis of serotype M18 group A Streptococcus strains associated with acute rheumatic fever outbreaks" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 99, no. 7, 2 April 2002 (2002-04-02), pages 4668-4673, XP002267116 ISSN: 0027-8424 SPYM18_0020 is 99,497% identical to present GAS5 over the whole sequence	1,24
P,X	DATABASE UniProt [Online] 23 November 2004 (2004-11-23), XP002367843 retrieved from EBI Database accession no. Q5XEL1 the whole document	1,24
X	-& BANKS D J ET AL: "PROGRESS TOWARD CHARACTERIZATION OF THE GROUP A STREPTOCOCCUS METAGENOME: COMPLETE GENOME SEQUENCE OF A MACROLIDE-RESISTANT SEROTYPE M6 STRAIN" JOURNAL OF INFECTIOUS DISEASES, CHICAGO, IL, US, vol. 190, no. 4, 15 August 2004 (2004-08-15), pages 727-738, XP008047099 ISSN: 0022-1899 M6_SPY0017 is 99,497% identical to present GAS5 over the whole sequence	1,24
A	FERRETTI J J ET AL: "Complete genome sequence of an M1 strain of Streptococcus pyogenes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 98, no. 8, 10 April 2001 (2001-04-10), pages 4658-4663, XP002168716 ISSN: 0027-8424	
A	OLIVE C ET AL: "Protection of mice from group A streptococcal infection by intranasal immunisation with a peptide vaccine that contains a conserved M protein B cell epitope and lacks a T cell autoepitope" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 20, no. 21-22, 21 June 2002 (2002-06-21), pages 2816-2825, XP004357806 ISSN: 0264-410X	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/036009

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 37 and 38 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-40 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Inventions 1-68: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 2 in the apparition order in said Table 2, i.e. GAS antigen 5, 6, 18, 22, 23, 25, 29, 30, 36, 49, 56, 60, 62, 63, 65, 67, 68, 69, 74, 75, 76, 77, 78, 81, 82, 85, 86, 89, 91, 92, 93, 94, 96, 97, 98, 99, 100, 101, 103, 104, 105, 108, 123, 131, 142, 143, 158, 165, 166, 175, 178, 179, 187, 188, 190, 195, 205, 206, 207, 218, 219, 242, 249, 271, 291, 327, 380, 685, respectively, as well as to fragments and homologues of said sequences (e.g. GRAB precursor and SPs1285 of Table 11) and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 69-73: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 3 not mentioned in Table 2, in the apparition order in said Table 3, i.e. GAS 73, 74, 109, 129, 130, respectively, as well as to fragments and homologues of said sequence and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Invention 74: claims 1-40 (all partially)

Subject-matter relating to one of the GAS40 protein, in native form or as fusion protein as found in Table 4A-R as well as to fragments and homologues thereof (e.g. as found in Table 5), and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 75-137: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 7 not previously mentioned, in the apparition order in said Table 7, i.e. GAS 4, 15, 16, 24, 54, 57, 64, 72, 84, 102, 152, 157, 163, 168, 177, 191, 192, 193, 194, 198, 201, 224, 251, 259, 262, 264, 268, 277, 282, 299, 382, 405, 406, 425, 433, 460, 469, 493, 500, 545, 558, 587, 645, 650, 362-1, SPY0080a, 0272, 0461, 0611, 0717, 0792, 1029, 1073, 1260, 1613, 1835, 2005, 2093, 2178, GAS45, SPY0047, 0127, 0686, respectively, as well as to fragments and homologues thereof (e.g. M protein type 3 and C5A peptidase precursor of Table 11) and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 138-160: claims 1-40 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Subject-matter relating to one of the GAS proteins listed in Table 8 not previously mentioned, in the apparition order in said Table 8, i.e. GAS10, 83, 160, 284, 286, 292, 396, SPY0053, 0056, 0063, 0069, 0098, 0666, 0688, 0913, 1200, 1281, 1721, 1750, 1805, 2070, 2092 and g-21909751, respectively, as well as to fragments and homologues thereof and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 161-162: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 9 not mentioned previously, in the apparition order in said Table 9, i.e. NT01SP0246 and GAS309, respectively, as well as to fragments and homologues thereof and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 163-165: claims 1-40 (all partially)

Subject-matter relating to GAS proteins listed in Table 11 not mentioned previously in apparition order in said Table 11, i.e. SPY1664, GAS149 and SPY0861, respectively, and to fragments and homologues thereof (e.g. putative penicillin binding proteins 2X, putative large conductance mechanosensitive channel and hypothetical protein SPs1270 found in Table 11), and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 166-180: claims 1-40 (all partially)

Subject-matter relating to the proteins listed in Table 12 considering the references given.

Inventions 181-207: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 15 not mentioned previously in the apparition order in said Table 15, i.e. GAS35, 414, 426, 434, 437, 438, 439, 461, 465, 472, 473, 475, 477, 478, 495, 538, 543, 553, 561, 576, 577, 591, 592, 636, 643, 649, 663, respectively, as well as to fragments and homologues thereof, the encoding nuceic acid sequences and antibodies directed to said proteins.

Inventions 208-236: claims 1-40 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Subject-matter relating to the GAS proteins listed in Table 16 not mentioned previously in apparition order of said Table 16, i.e. GAS42, GAS95, M30098, M3_0100, M3_0102, M3_0104, SPs0106, GAS130, GAS137, M6_0157, M6_0159, GAS159, M6_0160, GAS217, 220, 290, 294, 384, 504, 509, 511, 527, 529, 533, 680, 19224134, 19224135, 19224137, 19224141, respectively, as well as to fragments and homologues thereof, the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 237-272: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins not previously mentioned in the Tables but cited in the claims, in the apparition order in said claims, i.e. NT01SP0102, NT01SP0908 (SPY1111), NT01SP0182 (SPY0216), GAS70, 421, 428, 457, 474, 486, 492, 494, 535, 540, 560, 564, 565, 574, 579, 586, 607, 609, 625, 626, 640, 653, 657, 39, 58, 236, 366, 372, 389, Mprotein, SagA, Sfb1, Shp, respectively, as well as to fragments and homologues thereof, the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 273-275: claim 36, partially

Subject-matter relating to the use of an antibody in the manufacture of a medicament for treating S. pyrogenes infection, wherein the antibody specifically binds to a surface-exposed GAS antigen which is shorter by at least one amino acid than a GAS protein listed in Table 1 which was not previously mentioned, i.e. GAS41, GAS183 and GAS202, respectively.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2005/036009

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004078907 A	16-09-2004	AU 2004218284 A1	16-09-2004
		CA 2517518 A1	16-09-2004

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REVISED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
WO 2006/042027 A3

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A61K 39/09 (2006.01) *A61K 39/40* (2006.01)

(21) International Application Number:
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(22) International Filing Date: 11 October 2005 (11.10.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

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60/652,736	15 February 2005 (15.02.2005)	US
60/701,121	21 July 2005 (21.07.2005)	US
60/705,209	4 August 2005 (04.08.2005)	US

(71) Applicant *(for all designated States except US)*: **CHIRON CORPORATION** [US/US]; 4560 Horton Street, Emeryville, Ca 94608 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BENSI, Giuliano** [IT/IT]; Via Fiorentina 1, I-53100 Siena (IT). **GRANDI, Guido** [IT/IT]; Via Fiorentina, 1, I-53100 Siena (IT). **NORAIS, Nathalie** [—/US]; c/o Chiron Corpopration, 4560 Horton Street, Emeryville, CA 94608 (US). **ORTEGA, Manuel, Rodriguez, J.** [—/US]; C/o Chiron Corporation, 4560 Horton Street, Emeryville, CA 94662 (US).

(74) Agents: **HEMMENDINGER, Lisa, M.** et al.; Banner & Witcoff, Ltd., 1001 G Street, N.W.., 11th Floor, Washington, D.C. 20001-4597 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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2 November 2006

(15) Information about Correction:
see PCT Gazette No. 44/2006 of 2 November 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: IMMUNOGENIC AND THERAPEUTIC COMPOSITIONS *FOR STREPTOCOCCUS PYOGENES*

(57) Abstract: Group A streptococcal (GAS) antigens useful for providing immunity against *pyogenes* infection.

WO 2006/042027 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/036009

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/315 A61K39/09 A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 2006	13. 09. 2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mauhin, V

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/078907 A (INTERCELL AG; MEINKE, ANDREAS; NAGY, ESZTER; WINKLER, BIRGIT; GELBMANN) 16 September 2004 (2004-09-16) page 9, line 49 - page 10, line 21 page 12, line 15 - line 16 page 14, line 3 - line 4 page 22, line 32 - line 39 page 24, line 4 - line 5 page 31, line 31 - page 32, line 22 page 35, line 50 - page 37, line 28 page 39, line 16 - line 30 page 40, line 8 - line 22 spy0019 of SEQ. ID. No. 152 is 100% identical to present GAS5 (SEQ. ID. No. 2). Specific epitopes are disclosed. The encoding DNA of SEQ. ID. No. 2 is 100% identical to present GAS5 (SEQ. ID. No. 651) figure 4a; example 5; tables 1,3	1-40

X	DATABASE UniProt [Online] 1 October 2002 (2002-10-01), XP002367841 retrieved from EBI Database accession no. Q8P318 the whole document	1,24
X	-& NAKAGAWA ICHIRO ET AL: "Genome sequence of an M3 strain of Streptococcus pyogenes reveals a large-scale genomic rearrangement in invasive strains and new insights into phage evolution." GENOME RESEARCH, vol. 13, no. 6a, June 2003 (2003-06), pages 1042-1055, XP002367632 ISSN: 1088-9051 protein SPS0015 SPYM3_0014 is 99,497% identical to present GAS5 over the whole sequence.	1,24

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/036009

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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P,X	DATABASE UniProt [Online] 23 November 2004 (2004-11-23), XP002367843 retrieved from EBI Database accession no. Q5XEL1 the whole document	1,24
X	-& BANKS D J ET AL: "PROGRESS TOWARD CHARACTERIZATION OF THE GROUP A STREPTOCOCCUS METAGENOME: COMPLETE GENOME SEQUENCE OF A MACROLIDE-RESISTANT SEROTYPE M6 STRAIN" JOURNAL OF INFECTIOUS DISEASES, CHICAGO, IL, US, vol. 190, no. 4, 15 August 2004 (2004-08-15), pages 727-738, XP008047099 ISSN: 0022-1899 M6_SPY0017 is 99,497% identical to present GAS5 over the whole sequence	1,24
A	FERRETTI J J ET AL: "Complete genome sequence of an M1 strain of Streptococcus pyogenes" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 98, no. 8, 10 April 2001 (2001-04-10), pages 4658-4663, XP002168716 ISSN: 0027-8424	
A	OLIVE C ET AL: "Protection of mice from group A streptococcal infection by intranasal immunisation with a peptide vaccine that contains a conserved M protein B cell epitope and lacks a T cell autoepitope" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 20, no. 21-22, 21 June 2002 (2002-06-21), pages 2816-2825, XP004357806 ISSN: 0264-410X	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/036009

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-40 (all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Inventions 1-68: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 2 in the apparition order in said Table 2, i.e. GAS antigen 5, 6, 18, 22, 23, 25, 29, 30, 36, 49, 56, 60, 62, 63, 65, 67, 68, 69, 74, 75, 76, 77, 78, 81, 82, 85, 86, 89, 91, 92, 93, 94, 96, 97, 98, 99, 100, 101, 103, 104, 105, 108, 123, 131, 142, 143, 158, 165, 166, 175, 178, 179, 187, 188, 190, 195, 205, 206, 207, 218, 219, 242, 249, 271, 291, 327, 380, 685, respectively, as well as to fragments and homologues of said sequences (e.g. GRAB precursor and SPs1285 of Table 11) and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 69-73: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 3 not mentioned in Table 2, in the apparition order in said Table 3, i.e. GAS 73, 74, 109, 129, 130, respectively, as well as to fragments and homologues of said sequence and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Invention 74: claims 1-40 (all partially)

Subject-matter relating to one of the GAS40 protein, in native form or as fusion protein as found in Table 4A-R as well as to fragments and homologues thereof (e.g. as found in Table 5), and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 75-137: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 7 not previously mentioned, in the apparition order in said Table 7, i.e. GAS 4, 15, 16, 24, 54, 57, 64, 72, 84, 102, 152, 157, 163, 168, 177, 191, 192, 193, 194, 198, 201, 224, 251, 259, 262, 264, 268, 277, 282, 299, 382, 405, 406, 425, 433, 460, 469, 493, 500, 545, 558, 587, 645, 650, 362-1, SPY0080a, 0272, 0461, 0611, 0717, 0792, 1029, 1073, 1260, 1613, 1835, 2005, 2093, 2178, GAS45, SPY0047, 0127, 0686, respectively, as well as to fragments and homologues thereof (e.g. M protein type 3 and C5A peptidase precursor of Table 11) and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 138-160: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 8 not previously mentioned, in the apparition order in said Table 8, i.e. GAS10, 83, 160, 284, 286, 292, 396, SPY0053, 0056, 0063, 0069, 0098, 0666, 0688, 0913, 1200, 1281, 1721, 1750, 1805, 2070, 2092 and g-21909751, respectively, as well as to fragments and homologues thereof and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 161-162: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 9 not mentioned previously, in the apparition order in said Table 9, i.e. NT01SP0246 and GAS309, respectively, as well as to fragments and homologues thereof and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 163-165: claims 1-40 (all partially)

Subject-matter relating to GAS proteins listed in Table 11 not mentioned previously in apparition order in said Table 11, i.e. SPY1664, GAS149 and SPY0861, respectively, and to fragments and homologues thereof (e.g. putative penicillin binding proteins 2X, putative large conductance mechanosensitive channel and hypothetical protein SPs1270 found in Table 11), and to the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 166-180: claims 1-40 (all partially)

Subject-matter relating to the proteins listed in Table 12 considering the references given.

Inventions 181-207: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins listed in Table 15 not mentioned previously in the apparition order in said Table 15, i.e. GAS35, 414, 426, 434, 437, 438, 439, 461, 465, 472, 473, 475, 477, 478, 495, 538, 543, 553, 561, 576, 577, 591, 592, 636, 643, 649, 663, respectively, as well as to fragments and homologues thereof, the encoding nuceic acid sequences and antibodies directed to said proteins.

Inventions 208-236: claims 1-40 (all partially)

Subject-matter relating to the GAS proteins listed in Table 16 not mentioned previously in apparition order of said Table 16, i.e. GAS42, GAS95, M30098, M3_0100, M3_0102, M3_0104, SPs0106, GAS130, GAS137, M6_0157, M6_0159, GAS159, M6_0160, GAS217, 220, 290, 294, 384, 504, 509, 511, 527, 529, 533, 680, 19224134, 19224135, 19224137, 19224141, respectively, as well as to fragments and homologues thereof, the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 237-272: claims 1-40 (all partially)

Subject-matter relating to one of the GAS proteins not previously mentioned in the Tables but cited in the claims, in the apparition order in said claims, i.e. NT01SP0102, NT01SP0908 (SPY1111), NT01SP0182 (SPY0216), GAS70, 421, 428, 457, 474, 486, 492, 494, 535, 540, 560, 564, 565, 574, 579, 586, 607, 609, 625, 626, 640, 653, 657, 39, 58, 236, 366, 372, 389, Mprotein, SagA, Sfb1, Shp, respectively, as well as to fragments and homologues thereof, the encoding nucleic acid sequences and antibodies directed to said proteins.

Inventions 273-275: claim 36, partially

Subject-matter relating to the use of an antibody in the manufacture of a medicament for treating S. pyrogenes infection, wherein the antibody specifically binds to a surface-exposed GAS antigen which is shorter by at least one amino acid than a GAS protein listed in Table 1 which was not previously mentioned, i.e. GAS41, GAS183 and GAS202, respectively.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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